FACTORS CONTROLLING THE DISTRIBUTION AND ABUNDANCE OF A FILTER-FEEDING MAYFLY, *COLOBURISCUS HUMERALIS*, IN NEW ZEALAND STREAMS

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FOR
GRAHAM IRA MCNABB

CONTENTS

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ABSTRACT

The influence of abiotic and biotic factors on the distribution and abundance of filter-feeding invertebrates has not been well studied. This study examined the effect of predatory fish and invertebrates, food supply and abiotic factors on the distribution of *Coloburiscus humeralis*, a filter feeding mayfly, in streams near Cass, Hanmer and on Banks Peninsula, New Zealand. Observations of the feeding behaviour and examinations of mouthpart morphology using scanning electron microscopy together with a distribution survey, channel experiment and behavioural studies were used to elucidate patterns.

Stability was the major factor controlling the distribution and abundance of *Coloburiscus* in streams and the density of nymphs was reduced by simulated bed disturbance in stream channels. Higher abundances of nymphs were associated with more stable streams. Other abiotic factors affecting the distribution of *Coloburiscus* were also connected with stream stability (i.e. current velocity and substrate size). Predatory fish did not influence the distribution or abundance of *Coloburiscus* in streams or stream channels and no nymphs were found in the gut contents of trout or galaxiids in the channel experiment. However, native galaxiids affected the feeding behaviour of nymphs, by decreasing the amount found in the guts of *Coloburiscus*. Anti-predator defences, such as the cerci, may reduce the risk of predation but predatory galaxiids still affect the feeding behaviour of *Coloburiscus* nymphs.

The results of this study imply there is a trade-off between vulnerability of *Coloburiscus* to predation and susceptibility to disturbance. Predatory fish had no effect on the distribution or abundance of *Coloburiscus* probably because they are

morphologically defended and move very little but *Coloburiscus* nymphs were not found in unstable streams. The high cost of investing in anti-predator defences results in increased vulnerability to disturbance because they are not mobile enough to seek refuge.

CHAPTER 1

INTRODUCTION

CHAPTER ONE

Introduction

"Ecology is not a science with a simple linear structure: everything affects everything else" (pg vii, Begon, Harper and Townsend, 1996). In terrestrial and aquatic ecosystems, both biotic and abiotic factors influence community structure and population dynamics. One of the most important challenges within ecology is to measure the strength of these factors, examine the interaction between them and thus explain patterns in the distribution and abundance of plant and animal species (Hunter and Price, 1992).

Various studies have shown that abiotic factors influence the role of biotic factors. For example, in benthic stream communities increased habitat complexity can lead to increased niche diversity and more refugia from predatory fish for prey species (Dahl and Greenberg, 1998). Seasonal variation also changes the way biomass is distributed among species and the assembly of species in a food web (Thompson and Townsend, 1999).

Biotic factors can be divided into two groups depending on the kind of control they have on the food web. The structure of lower trophic levels may depend directly or indirectly on higher trophic levels (Menge, 1992); defined as "top-down control". "Bottom-up control" refers to the direct or indirect dependence of community structure on factors at lower trophic levels, for example resource supply (Menge, 1992). The importance of these two forms of control has been debated widely (Power, 1990, 1992; Polis, 1994), with some studies proposing an integration of the two forces (McQueen et al., 1989; Hunter and Price, 1992; Strong, 1992). In many

cases, a variety of biotic and abiotic factors will regulate the relative strengths of predator control and resource limitation of populations (Hunter and Price, 1992).

The influence of abiotic and biotic factors on stream invertebrates has concentrated on grazing species and autotrophic systems (see McIntosh, 2000). Conclusions made from these studies have been used to infer relationships between abiotic and biotic factors and other functional feeding groups. Stream invertebrates may respond differently to abiotic and biotic factors depending on their feeding habits and position in the food chain. Grazing invertebrate species which feed on the tops of rocks have a greater risk of predation than shredding species whose food source may be under or between rocks (e.g., Cowan and Peckarsky, 1994; Cummins et al., 1989). Filter-feeding species rely on current to obtain food whereas predatory species may be more indifferent to the flow regime.

In New Zealand, the filter-feeding mayfly *Coloburiscus humeralis* feeds on a current-borne food supply that may be affected by biotic or abiotic factors, or a combination of both. As suggested above, changes in velocity may be more important for *Coloburiscus* than for grazing invertebrates. Likewise, risk of predation may be lower for *Coloburiscus* than other species of mayfly because of its preferred habitat under rocks and in crevices. Abiotic and biotic factors may also jointly affect *Coloburiscus* nymphs, for example the effect of velocity on food supply. In this study, I have investigated some biotic and abiotic influences on the distribution, abundance, morphology and feeding of *Coloburiscus humeralis*.

1.1 *Coloburiscus humeralis* – A filter-feeding mayfly

The genus *Coloburiscus* (family Coloburiscidae) contains two described species, *C. tonnoiri* Lestage and *C. humeralis* (Walker), however *C. tonnoiri* may not be distinct from *C. humeralis* (Winterbourn, Gregson and Dolphin, 2000). *Coloburiscus* is relatively widespread around New Zealand and its life history and production have been studied (McLean, 1967; Towns, 1987; Chadderton, 1988; Harding and Winterbourn, 1993). However, little is known about the factors that control the local distribution, abundance and feeding behaviour of this mayfly species.

Wisely (1961, 1962, and 1965) documented the early life history, ecology and distribution of nymphs, and the morphology and anatomy of adult *Coloburiscus* in a series of three papers. In one of those studies, which included observations of *Coloburiscus* feeding in the laboratory, he found that the hairs or setae on the prothoracic legs were used to capture food particles from the water column (Wisely, 1961). These particles of fine particulate organic matter (FPOM) are referred to as seston.

Coloburiscus is a filter feeder, so it relies on particles entering steams being small or on other invertebrates in the stream to breakdown leaves or coarse particulate organic matter (CPOM) into a manageable size that it can consume. Shredders and detritivores with the aid of microorganisms break down CPOM in streams (Fig. 1). The supply of seston available to *Coloburiscus* is likely to be determined by catchment runoff, bank erosion, stream current, physical abrasion and the presence of invertebrates and fish in the stream. The movement of invertebrates and fish in a stream as well as erosion by the current will suspend benthic FPOM in the water column (Fig. 1.1) (Zanetell and Peckarsky, 1996; Statzner, 1981).

Within every food web there are also predators which may affect these processes and interactions. Predators may consume *Coloburiscus* directly or affect other invertebrates indirectly (Fig. 1.1) thereby causing changes in the amount of food available to *Coloburiscus*. This is discussed further in section 1.3. The rest of this chapter deals with the effect of abiotic and biotic factors on the distribution, abundance and behaviour of *Coloburiscus humeralis*.

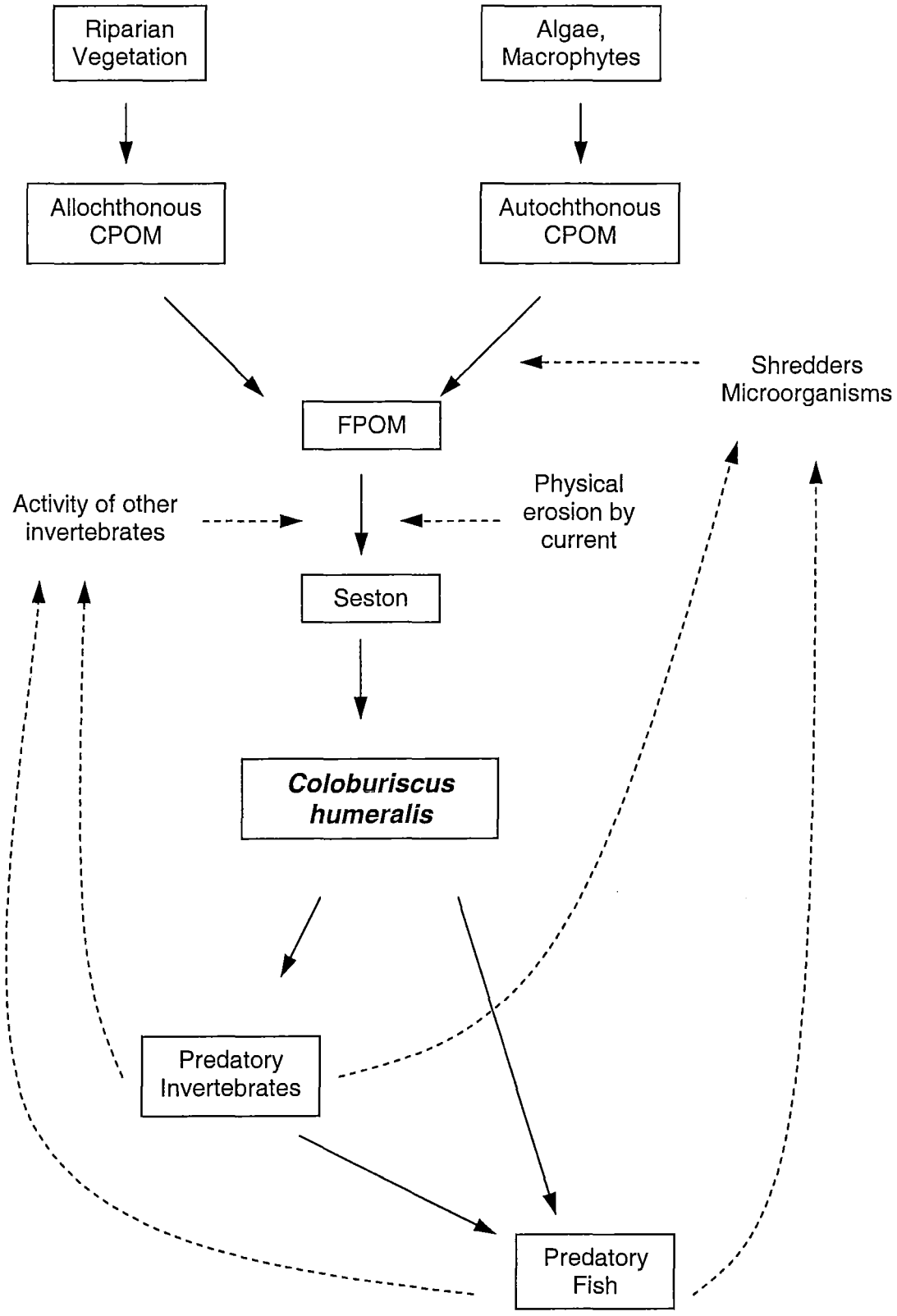

Figure 1.1: Hypothetical relationship between *Coloburiscus humeralis*, predatory fish and other invertebrate species in a stream. Dashed line indicates possible "effects"; Solid line indicates energy path.

1.2 Abiotic Factors

In ecological studies of streams, it is important to examine the role of abiotic factors. For example temperature, pH, oxygen concentration, velocity and stability have been found to influence the distribution and abundance of stream invertebrates (Sweeney, 1978; Jowett and Richardson, 1990; Huryn, 1996). However, the response of different species will vary depending on their feeding mode, water quality and habitat requirements (e.g. Ciborowski, 1983; Englund, 1991; Voelz and Ward, 1996). In the next section I review the influence of some abiotic factors on stream invertebrates, particularly filter feeders.

1.2.1 Current Velocity

In streams and rivers, current velocity is one of the most important determinants of community structure. The linear unidirectional movement of water may determine food availability, habitat selection and movement of stream invertebrates (see Allan, 1995). Current velocity may also influence other abiotic factors such as oxygen concentration, substrate composition and temperature. For example, large nymphs of the New Zealand mayfly *Deleatidium* were found in faster velocities than smaller ones (Collier, 1994). This partitioning of size classes was thought to be associated with changes in oxygen requirements as *Deleatidium* nymphs grow larger (Collier, 1994). Jowett and Richardson (1990) also suggested that velocity preferences of *Deleatidium* and some caddisfly species may change with size or life stage. Observations suggest *Coloburiscus humeralis* also requires fast flowing water for respiration (Wisely, 1962).

The reliance of filter feeders on current velocity to obtain food means velocity is a particularly important factor influencing their distribution and abundance (see Wallace and Merritt, 1980). They use a variety of different methods, including nets and hairs to trap food particles from the water column. In faster velocities, there may be more opportunities for feeding because more water is passing through the nets or hairs of filter feeders. However, there may also be related costs. At very fast velocities (>50 – 60 $cm.s^{-1}$) there is more chance of being sweep away in the current

and more energy is used to maintain position on the substratum (Statzner, 1981). Moreover, in faster velocities these energy costs may exceed the benefits of greater food availability. Hydropsychid (Winterbourn and Harding, 1993) and *Simulium* (Malmqvist and Sackmann, 1996) larval densities increase with current velocity up to a certain threshold (hydropsychid: approx. 60cm.s^{-1}; *Simulium*: 53 cm.s^{-1}). However, in a Colorado stream, two species of filter-feeding caddisflies tended to avoid current-exposed areas as they became larger (Voelz and Ward, 1996).

Voelz and Ward (1996) proposed that the pattern of water movement over the substrate was more important than velocity for habitat selection by stream invertebrates, especially for filter feeders. Substrate surfaces may contribute to differences in current pattern, with rougher surfaces causing greater small scale variability in current velocity (Quinn and Hickey, 1990). This may lead to a greater range of microhabitats and therefore more refugia from predation (Quinn and Hickey, 1990), and may be particularly important for relatively immobile species of invertebrates. Cardinale et al. (2002) found that filter-feeding hydropsychid caddisflies in diverse habitats experienced higher water velocities through their nets and also exhibited a greater range in the size of nets. Increasing velocity led to an increase in consumption of seston.

Predation by fish or predatory invertebrates may also be affected by velocity. By using laboratory flumes, Malmqvist and Sackmann (1996) were able to manipulate the current velocity to which *Simulium* larvae were exposed, and also introduced a variety of predatory invertebrates. Optimum feeding occurred at intermediate current velocities (18.8 and 36.2 cms^{-1}) when predatory invertebrates were absent. However, when stoneflies, *Isoperla grammatica* and *Diura nanseni*, were present the *Simulium* larvae selected microhabitats in higher current velocities to avoid predation (Malmqvist and Sackmann, 1996). This decreased their risk of predation by predatory stoneflies but also reduced their feeding success. Similar interactions could affect the distribution of *Coloburiscus*, which may avoid areas where the risk of predation is high but food supply is low. Therefore, in this study I have examined the direct effects of abiotic factors on *Coloburiscus* and interactions with biotic variables.

1.2.2 Substrate

In New Zealand many invertebrate species have been termed generalist feeders (Winterbourn, 2000), with very few having a specialized feeding mode. In a review of invertebrate-substratum relationships, Death (2000) suggested that niche requirements should also be unspecialised because of this lack of specialization in feeding. However, there have been many studies overseas (Dahl and Greenberg, 1998) and in New Zealand (e.g., Quinn and Hickey, 1990 b; Jowett and Richardson, 1990), which demonstrate that invertebrates preferentially occupy certain substrates.

Substrate composition may reflect the stability of a stream. For example, larger substrates such as boulders or large cobbles are usually more stable than smaller substrates (e.g., gravel and sand). Increased substrate size has also been associated with higher standing crops of periphyton and coarse particulate organic matter (CPOM) (Quinn and Hickey, 1990). Invertebrate density and taxon richness were found to increase as substrate size increased from sand to cobble, but were lower on bedrock (Quinn and Hickey, 1990). Jowett and Richardson (1990) also showed that affinities for certain habitat variables (substrate, velocity and depth) occurred in some New Zealand invertebrate species. Preferences of different species may vary depending on feeding mode, water quality requirements or biotic interactions (Quinn and Hickey, 1990). *Simulium* and hydropsychid larvae filter-feed on the tops of rocks (Craig and Chance, 1982; Englund, 1991; Winterbourn and Harding, 1993; Harding, 1997; Miller et al., 1998) and larger substrates therefore may be important as stable feeding sites.

Coloburiscus humeralis is generally associated with the undersides of rocks, but still shows a preference for larger substrates (Quinn and Hickey, 1990). This may be related to the morphology of *Coloburiscus* rather than its position when feeding. The large cumbersome body and poor swimming ability of *Coloburiscus* may cause it to avoid the upper surfaces of rocks where the nymphs may be washed off. Campbell (1985) suggested that the gills of the morphologically similar *Coloburiscoides* were used to hold the nymph in position under rocks and in crevices. If this is also true for *Coloburiscus* then the gills may be used to maintain the position of nymphs under larger substrates.

Habitat heterogeneity may also affect invertebrate abundance and species richness (Scarsbrook and Townsend, 1993; Death, 1995). The abundance of "sedentary species", filter feeders and shredders was found to be higher in a more stable, heterogeneous stream compared to a more uniform, unstable stream which had a higher abundance of mobile species (Scarsbrook and Townsend, 1993). This may be the result of a greater number of potential habitat refuges in the more heterogeneous stream (see Power, 1992). However, Death (1995) found that substrate stability was more important than substrate heterogeneity in determining community structure. Enclosure experiments used in a Swedish creek to test the effects of substrate heterogeneity and predation on a benthic invertebrate species also indicated there was no effect of habitat heterogeneity (Dahl and Greenberg, 1998). These studies show that habitat heterogeneity has the potential to influence invertebrate communities, but the influence depends on the system and species present.

1.2.3 Temperature

Temperature is another important factor that influences both abiotic variables and stream organisms (e.g., Scrimgeour, 1991; Winterbourn and Harding, 1993; Petersen et al., 1999). Temperature was the main factor controlling the growth and emergence of the New Zealand mayfly *Deleatidium* (Huryn, 1996). It also controlled the developmental rate of eggs and nymphs of the North American mayfly, *Isonychia bicolor* (Sweeney, 1978).

Lower temperatures may affect the metabolic rate of stream invertebrates, for example changes in respiration rate of *I. bicolor* were associated with temperature (Sweeney, 1978). Lower metabolic rates may also result in a decrease in food processing ability. The nutritional value of food is determined by its composition, as well as the microorganisms present on the food (Anderson and Cummins, 1979). At lower temperatures the microorganisms may not be as efficient, and subsequently, the processing abilities of the invertebrate species may be reduced. Söderström (1988) found that food quality and temperature were significantly linked with growth rates of two *Parameletus* species. Therefore, interactions between food and abiotic factors are important to investigate.

Temperature was an important variable to consider in this study because it affects a variety of other factors. The growth of invertebrates, and therefore the length of time they spend in the stream, are controlled by temperature. If biotic factors, such as predation and food availability are considered, it is possible that fast growing individuals will avoid longer periods when they are at risk of predation (Peckarsky et al., 2002). Also, if variations in food levels occur, fast growing individuals may optimise the time spent in the stream and avoid periods when food levels are low. *Coloburiscus* is a large conspicuous mayfly, therefore higher temperatures may allow it to grow faster and avoid predation by fish. However, higher temperatures are associated with lower oxygen levels and because *Coloburiscus* is sensitive to low oxygen levels, lower temperatures may be preferred (Wisely, 1961).

1.3 Biotic Factors

Biotic factors may influence an organism directly or indirectly through the presence or activities of other organisms (e.g., competition and predation) (Allaby, 1994). Research investigating biotic interactions in streams has focused mainly on grazing invertebrates and species of predatory invertebrate or fish (Power, 1990; Peckarsky et al., 1993; McIntosh, 1995; McIntosh and Townsend, 1996. Consequently, this leaves large gaps in our knowledge of invertebrate species (e.g. detritivores, shredders or filter feeders) and their interactions with predators. In this study I examined the distribution, abundance and behaviour of the filter-feeding mayfly *Coloburiscus* with respect to biotic as well as abiotic factors.

1.3.1 Top-down control

Top-down control occurs when the structure (e.g., abundance and biomass) of lower trophic levels depends on the influence of organisms at higher trophic levels (Begon et al., 1996). Many studies have shown that this is important in stream communities (Power, 1990; Flecker and Townsend, 1994; McIntosh and Townsend,

1995, 1996). However, many other studies suggest that bottom-up control is more important, particularly for detritivorous insects (Wallace et al., 1999). These are discussed in the next section.

In New Zealand streams, top-down control has been demonstrated in streams containing both introduced trout and native galaxiids (Flecker and Townsend, 1994; McIntosh and Townsend, 1996; Huryn, 1998). Flecker and Townsend (1994) showed that trout caused a reduction in the abundance of grazing invertebrates and restricted their movements compared to those of grazers in control channels. This in turn increased the algal standing crop via a trophic cascade. However, the impact of galaxiids varied depending on the invertebrate species. The abundance of a grazing mayfly, *Deleatidium* and a filter-feeding dipteran, *Austrosimulium*, decreased in the presence of galaxiids, whereas the grazing caddisfly *Helicopsyche* increased in abundance (Flecker and Townsend, 1994).

In many studies of stream food webs, the influence of predatory fish and invertebrates on grazing invertebrates has been examined thoroughly (Power, 1990; Flecker, 1992; McIntosh and Townsend, 1996; Peckarsky, 1996). However, relatively little is known about the influence of predators on other functional feeding groups. Grazing mayflies (e.g., *Deleatidium*) feed on the tops of rocks, so it is not surprising that visually-feeding predatory fish like trout (McIntosh and Townsend, 1995) have such a large impact on their feeding behaviour. In contrast, many filter-feeding invertebrate species are found under stones or in gaps between rocks so fish predation may be reduced. However, one New Zealand filter feeder, *Austrosimulium,* is found on the tops of rocks and this may explain its response to the presence of galaxiids in the Flecker and Townsend (1994) study. Few studies have directly examined the effects of predation on filter feeders.

The large body size and clumsy swimming ability of *Coloburiscus* may make it more susceptible to predatory fish than are more streamlined mayfly species like *Nesameletus*. However, the preferred habitat of *Coloburiscus* is under rocks, and morphological adaptations, such as spines on the body, legs and cerci, may reduce the risk of predation by predatory fish and invertebrates. Moreover, *Coloburiscus* rarely drifts (see Cadwallader, 1975; Glova and Sagar, 1989). Therefore, it may not be vulnerable to predation by fish.

The risk of predation by fish or invertebrates may be reduced by the presence of morphological structures such as spines. Thus, the length of spines in some invertebrate species was found to increase in the presence of predators (Johansson and Samuelsson, 1994; Straile and Halbich, 2000). The presence of spines on *Ephemerella* nymphs, as well as their posturing behaviour, allows this mayfly species to avoid predatory fish successfully (Peckarsky, 1996). By comparison, other invertebrate species avoid any contact with predators by swimming away (Peckarsky, 1980; 1987). The presence of morphological characteristics on *Coloburiscus* is investigated in response to the presence of predatory species in Chapter Five.

If predation by fish and invertebrates has no effect on filter feeders due to the invertebrates' preferred habitat, feeding method or morphological defences, it is possible that predation on other invertebrates in the system may indirectly affect the filter-feeding species (Fig. 1). As discussed previously, filter feeders may rely heavily on other stream-dwelling species such as shredders because they breakdown CPOM into fine particulate organic matter (FPOM). The number of shredders, movement of invertebrates, or even the activities of predatory fish in a stream may determine the supply of FPOM. The movement of invertebrates and fish in a stream will transport benthic FPOM into the water column. Therefore, even if predatory fish are not directly consuming or altering the feeding behaviour of the filter-feeding species they may be affecting their food supply via their impact on other invertebrates (Fig. 1). This is investigated in Chapter Three.

1.3.2 Bottom-up control

Bottom-up control occurs when lower trophic levels influence community structure at higher trophic levels (Begon et al., 1996). In a food web, the removal of lower trophic levels (producers) will result in the collapse of higher trophic levels (consumers) (Hunter and Price, 1992).

Power (1992) suggested that the number of trophic levels in a food web determines the standing crop of primary producers, but the productivity of those producers is likely to constrain the number of trophic levels ultimately. For example,

in a three-tier food chain, the abundance of primary consumers will be suppressed by the predatory species (Fig. 1.2). This will allow increased abundance of the primary producer. However, in a four-tier food chain the secondary predator will suppress the primary predator, which in turn releases the primary consumer from predation and primary production will become limited (Fig. 1.2).

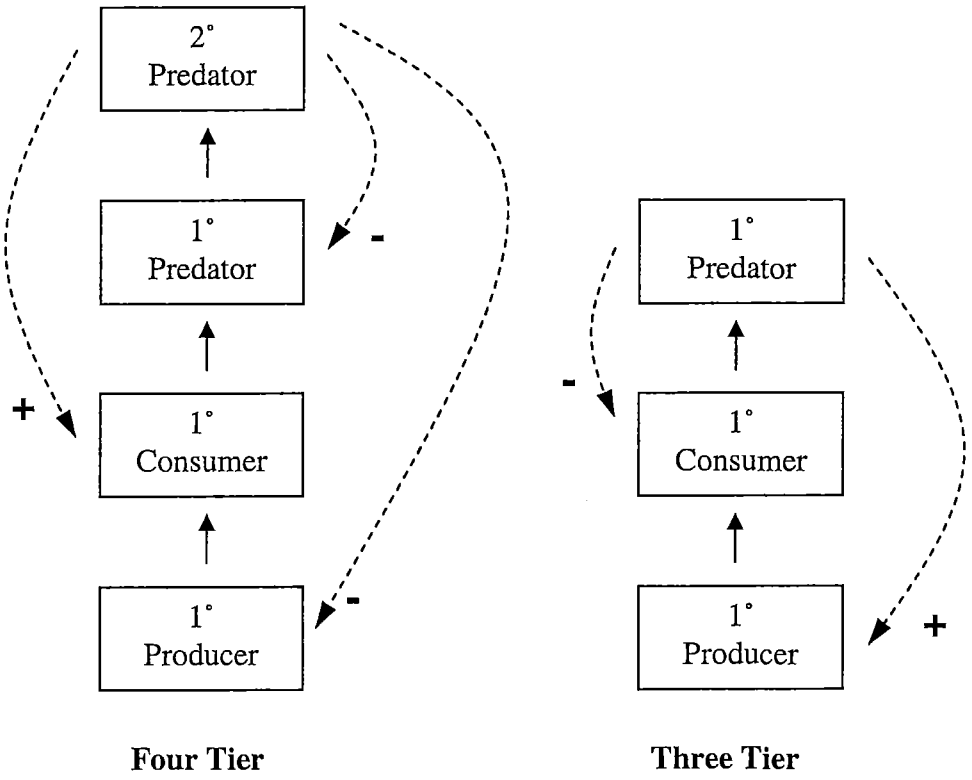

Figure 1.2: Hypothetical food chains differing in length. Solid arrow represents energy flow. Dashed arrow represents "effect": + positive, - negative.

Studies of bottom-up control in autotrophic food webs have predominated in freshwater ecosystems (Polis, 1994). However, detritus and allochthonous inputs can be very important, particularly in streams (Polis, 1994; Huryn, 1998; Wallace et al., 1999). Wallace et al. (1999) found that accumulated organic matter and allochthonous inputs maintained invertebrate community structure in a North Carolina stream. Biggs et al. (2000) suggested that the feeding behaviour of individual consumer species should be considered when investigating the effect of bottom-up

control in streams. Therefore, the degree to which stream invertebrates are influenced by bottom-up factors may be determined by their food source and related feeding behaviour. Polis (1994) also argued that food supply ultimately will control the survival, growth and reproduction of consumers. This is investigated in Chapter Three.

1.3.3 "Duality" of top-down and bottom-up control

The bottom-up: top-down model proposed by McQueen et al. (1989) predicts that "the maximum attainable biomass is determined by bottom-up forces but that the realized biomass is determined by the combined effects of top-down and bottom-up forces". For example, all invertebrate production was consumed in a New Zealand stream containing trout (Huryn, 1998). This in turn led to an increase in periphyton, which eventually became nutrient-limited. In many systems the biomass at each trophic level is determined by the combined effects of predation and nutrient availability (McQueen et al., 1989; Menge, 1992; Huryn, 1998).

In this study, the relative importance of abiotic and biotic factors was examined with respect to the distribution and abundance of *Coloburiscus humeralis* in streams in three regions of the South Island, New Zealand. Previous studies have shown that abiotic factors like temperature affect the growth of *Coloburiscus* (Harding and Winterbourn, 1993). I expanded on these studies and considered additional abiotic factors in order to determine reasons for patterns in the distribution and abundance of *Coloburiscus*. Biotic factors were also investigated with respect to the abundance of *Coloburiscus*, and associated research examined morphological and behavioural characteristics of nymphs.

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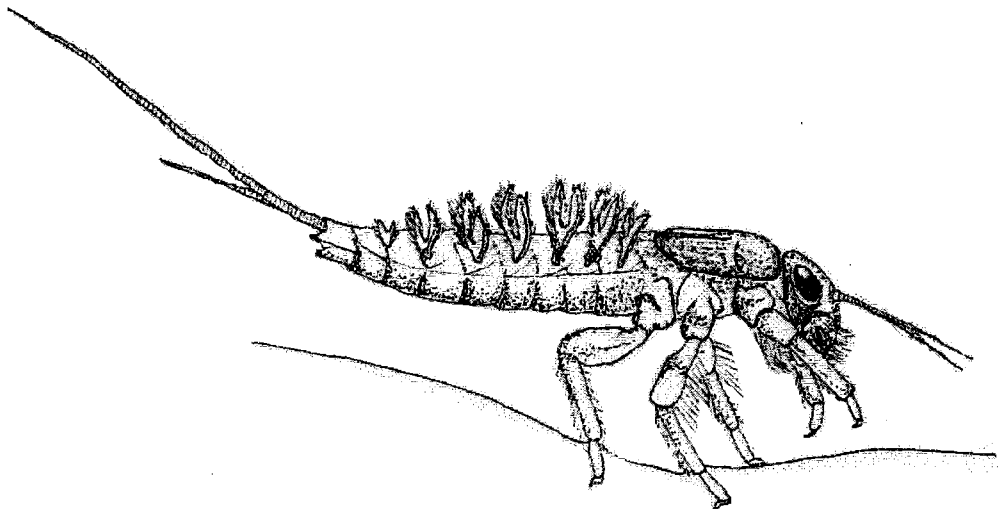


CHAPTER

CHAPTER TWO

Feeding Morphology of *Coloburiscus humeralis*

2.1 INTRODUCTION

Morphological studies are important tools for examining the ecology of an organism and can be used to gain information about the behaviour and ecology of invertebrates (e.g. Wisely, 1961; Pescador and Edmunds, 1994). For example, studies of stream invertebrate mouthparts provide information about feeding methods and an organism's role in the food web (see McShaffrey and McCafferty, 1986).

Eaton erected the genus *Coloburiscus* in 1887 for the species *Coloburiscus humeralis*, described earlier as *Palingenia humeralis* by Walker (1953). Early papers documented the morphology of *C. humeralis* in relation to habitat preferences (Phillips, 1930; Wisely, 1962). However, few studies have examined the structure and morphology of *C. humeralis*, extensively (Phillips, 1930; Wisely, 1965). The purpose of this chapter is to examine the functional morphology of the feeding structures of *C. humeralis* in relation to its classification as a filter feeder.

2.1.1 Feeding in Stream Invertebrates

Functional feeding groups classify species according to the way in which they obtain food (Cummins and Klug, 1979). Cummins and Klug (1979) identified five different functional feeding groups based on feeding mechanisms in stream invertebrates: (1) shredders, (2) collectors (gathering or filtering), (3) scrapers

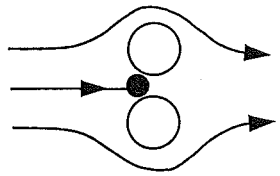
(grazers), (4) piercers and (5) predators. Subsequent classifications have differed somewhat. For example, Rounick, Winterbourn and Lyon (1982) classified New Zealand stream invertebrates as collector-browsers, omnivores, predators and shredders. These differences reflect the generalist feeding behaviour of many New Zealand stream invertebrates (Winterbourn, 2000).

Most mayfly species are collector-browsers or scrapers (Merritt and Cummins, 1996) but some are filter feeders (e.g. *Chloeon dipterum*: Brown, 1961; *Isonychia* spp.: Wallace and O'Hop, 1979; Braimah, 1987 a and b; Oligoneuriidae: Agnew, 1980; *Oligoneuriella marichuae*: Alba – Tercedor, 1990; *Oligoneuriella rhenana*: Elpers and Tomka, 1995), or predators. Morphological adaptations for filter-feeding in mayflies include setae on various appendages, including the maxillary and labial palps and the forelegs (Wallace and Merritt, 1980). Sticky secretions are used in conjunction with setae to capture food particles in some other filter feeding insects (Wallace and Merritt, 1980). For example, *Simulium* larvae produce a muco-substance which allows them to capture particles smaller than the pore size of their "sieve" mechanism (Ross and Craig, 1980). Comparable substances are uncommon in mayflies although a species of *Hexagenia* was found to have small spheres of mucus on its forelegs and mouthparts (Ross and Craig, 1980). Although larvae of *Hexagenia* are burrowers classified as collectors and gatherers, it is possible that some species filter at the mouth of the burrow (Merritt and Cummins, 1996).

Three approaches have been used to determine the function of feeding appendages in stream invertebrates (Palmer, 1998). "Morpho-behavioural" studies relate morphological structures to behavioural observations to explain the function of feeding structures. Observations of behaviour are helpful to determine the process of feeding. McShaffrey and McCafferty (1986) used small observation cells in conjunction with a specialized microscope and video camera to examine the behaviour of the collector-grazer *Stenacron interpunctatum.* This information was combined with anatomical studies using scanning electron microscopy (S E M) to determine structural details of feeding structures and to infer the process of feeding. Other studies have combined investigations of feeding behaviour and morphology to further understand feeding processes (Brown, 1961; Wallace and O'Hop, 1979).

A "morpho-mechanistic" approach has been used successfully to determine the function of feeding structures in several filter-feeding invertebrates (e.g. Ross and

Craig, 1980; Craig and Chance, 1982; Braimah, 1987a, b). The principal mechanisms used for particle capture by filter feeders were outlined by Rubenstein and Koehl (1977) and summarised by Vogel (1994). They include sieving, "direct interception", "inertial impaction", "gravitational deposition" and "diffusional deposition" (pp 358 - Vogel, 1994) (Fig. 2.1). The last four mechanisms assume that the food particle is smaller than the mesh size of the filtering structure. The food particle is "attracted" to the filter feeding structure by one of the following processes: current path (Fig 2.1b), inertia (Fig. 2.1c), gravitation (Fig. 2.1d) or random particle movement (Fig. 2.13e) (see summary in Vogel, 1994).

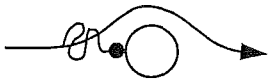


a) sieving

b) direct interception

c) inertial impaction

d) gravitational deposition



e) diffusional deposition

Figure 2.1: Five possible feeding mechanisms for suspension feeders. Arrows indicate movement of current: closed circle - food particle; Open circle - feeding structure (adapted from Vogel, 1994).

Braimah (1987a) evaluated these proposed mechanisms in his study of food particle capture in a blackfly, *Simulium bivittatum,* and the mayfly *Isonychia campestris*. He found that direct interception and diffusive deposition were the main mechanisms involved in capturing small food particles in these species (Fig. 2.1a, b). Direct interception involves the food particle passing "within a particle radius of the

collecting surface" (p 358 - Vogel, 1994). In contrast, diffusive deposition involves random movement of food particles in the current (Vogel, 1994). Patterns of flow around feeding structures have also been used to explain filter feeding in invertebrate species (Craig and Chance, 1982; Braimah, 1987b). The occurrence of laminar flow and a low Reynolds number (greater viscosity) facilitated filter feeding in *Simulium* larvae and *Isonychia campestris*.

The third approach used to determine the function of feeding structures is "morpho-ecological". Morphological observations of the feeding structures were used by Palmer to differentiate between species of *Simulium* larvae from different habitats (Palmer, 1998). This approach differs from the first two, in that it relates morphological characteristics to ecological principles, rather than specific function of the mouthparts.

Ephemeropteran larvae have evolved many different structures to exploit food resources (Elpers and Tomka, 1995), but most are specializations of the mouthparts (e.g. Brown, 1961; Craig and Chance, 1982). Using knowledge of these structures, as well as behavioural and dietary information, it is possible to classify species into functional feeding groups. *Coloburiscus humeralis* has been referred to as a filter feeder in many studies (e.g. Wisely, 1961; Rounick, Winterbourn and Lyon, 1982). This classification was based on early observations by Wisely (1961, 1962) and other authors (Phillips, 1930; Pendergrast and Cowley, 1966). The purpose of this chapter is to examine the feeding appendages and behaviour of *Coloburiscus humeralis* with respect to function using a "morpho-behavioural" approach in order to make inferences about the feeding of this mayfly.

2.2 METHODS

2.2.1 Feeding Behaviour

Coloburiscus humeralis larvae were collected from an unnamed fishless stream near Little River, on Banks Peninsula (see Chapter Three for details) using a 0.0625m^2 Surber sampler. Each stone within the sampling area was lifted and *Coloburiscus* larvae were removed gently until approximately one hundred

individuals were collected. The larvae were transported in a large plastic bucket equipped with a bubbler to keep the water oxygenated. In the laboratory, the mayflies were kept in an aquarium (40 cm (length) x 25 cm (height) x 25 cm (width)) with rocks from the collection site, and a water current was produced using bubblers. Water temperature was kept at approximately 10°C by placing the aquarium in an "ice bath".

Observations of *Coloburiscus* nymphs feeding were made with a video camera (Panasonic WV-BP 550) in a small aquarium (20 cm (l) x 15 cm (h) x 15 cm (w)) kept in a dark room. Fibre optic lights were used during the observation period to illuminate individuals. Small stones and CPOM were placed in the aquarium to simulate a natural environment. Five individuals were observed for a period of one hour. The tapes were reviewed later and behaviours observed were described.

2.2.2 Scanning Electron Microscopy

Whole specimens were dehydrated in a graded alcohol series (50, 70, 80, 90, 95 and 100% ethanol) for a minimum of one hour per solution. The larvae were then transferred to fresh 100% ethanol overnight. The following day, animals were transferred to amyl acetate (AA) at room temperature in a four step series (25% AA + 75% absolute ethanol; 50% AA + 50% abs. ethanol; 75% AA + 25% abs. ethanol; 100% AA), before being placed in 100% amyl acetate overnight.

The heads of three individuals were removed to enable examination of the mouthparts. The labial and maxillary palps, mandibles and hypopharynx were removed from one individual and mounted separately. Several other individuals were left intact so the inter-relationships of mouthparts and other structures could be examined.

All specimens were dried using a liquid CO_2 critical point drier and were mounted on aluminium stubs with carbon conductive paint. Each stub was sputter coated with 50 nm gold in a vacuum desiccator. Examination of the stubs was carried out using a scanning electron microscope (S E M) at magnifications up to 8000 x (18 kV).

3.3 RESULTS AND DISCUSSION

2.3.1 Feeding Behaviour

Larvae of *Coloburiscus humeralis* feed on fine particulate organic matter collected from the water column (seston) using the first pair of legs (forelegs) as a "sieve". My observations indicate that the second pair of legs was also used occasionally to capture food particles. Legs not used for feeding hold the substrate. The forelegs were held motionless in front of the head and body for 30 seconds to two minutes (Fig. 2.2) and were then brought to the mouth where the maxillary and labial palps removed small food particles (Fig. 2.3 e). The labial palps move forward (Fig. 2.3 e) in a circular motion (Fig. 2.3 a, b), followed by lateral movements of the maxillary palps (Fig. 2.3c). The mouthparts then came back to the resting position (Fig. 2.3 d). Feeding processes involving the mandibles and hypopharynx were hard to observe. Scanning electron microscopy was used to investigate the mouthparts further and infer processes and mechanisms involved during feeding.

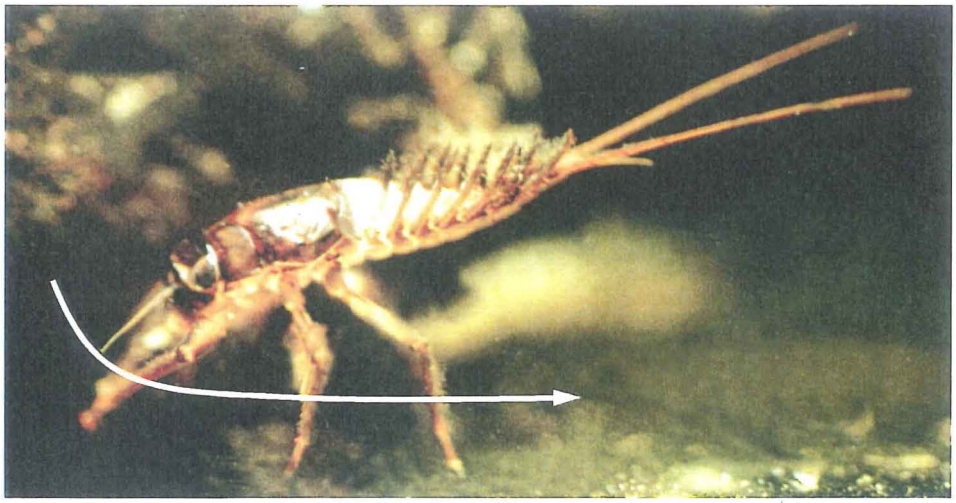


Figure 2.2: The forelegs of *Coloburiscus* nymphs are held off the substrate to enable particle capture. Arrow indicates current direction.

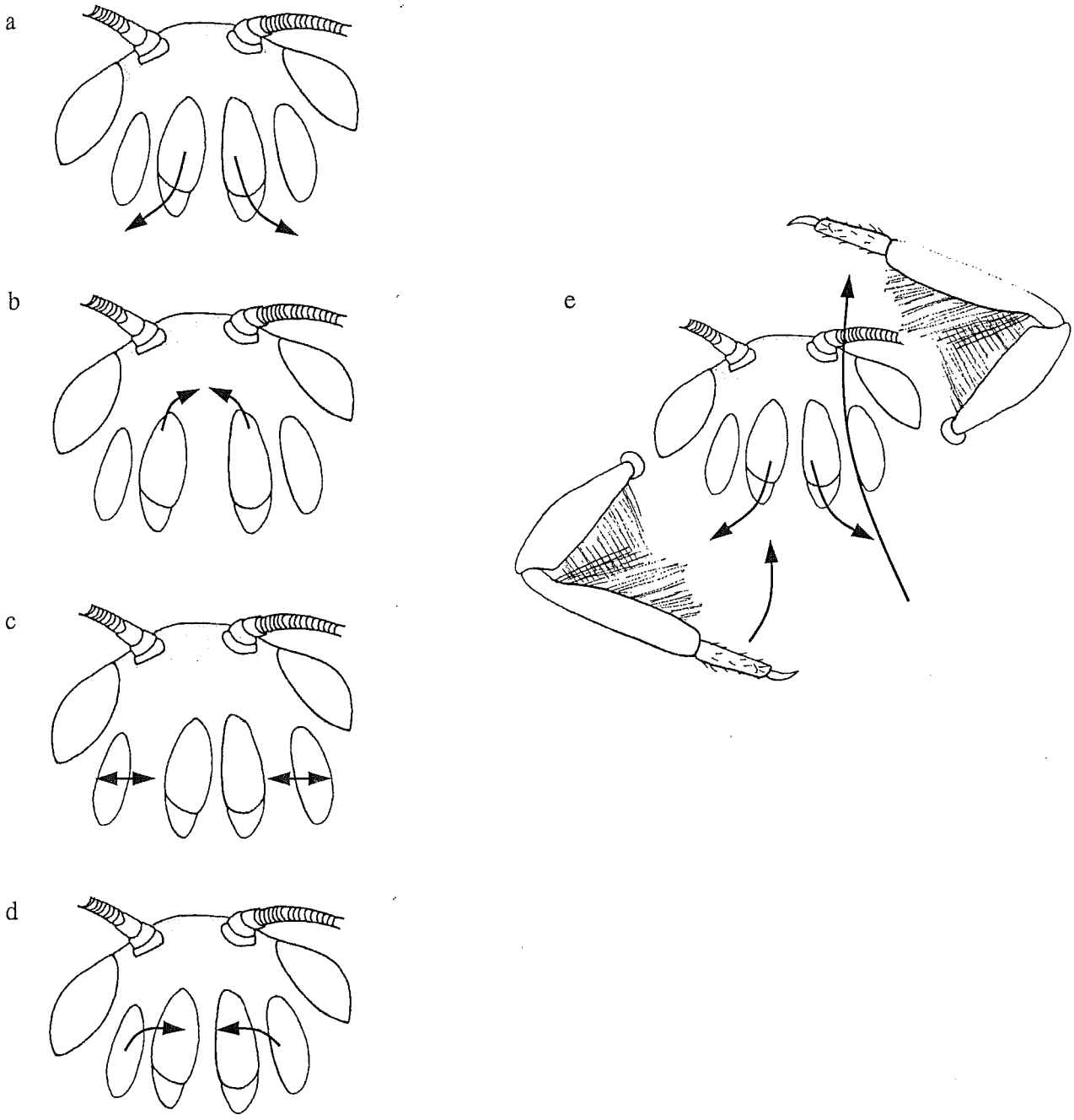


Figure 2.3: Diagrammatic representation of feeding in *Coloburiscus humeralis* nymphs. Labial palps open and move in a circular motion (a and b), followed by the maxillary palps which move laterally (c), then return to the resting position (d). The forelegs bring food particles to the mouth and are cleaned, alternately (e).

2.3.2 Scanning Electron Microscopy

The first two pairs of legs are the main appendages used for particle capture (Fig. 2.4). When the tibia and femur are positioned at approximately 90° to each other the hairs on their surfaces effectively form a net. There are two kinds of hairs on the tibiae of the forelegs of *Coloburiscus* nymphs (Fig. 2.5a). The longer hairs have a double row of microtrichia (Fig. 2.5b) and the smaller hairs have four rows of microtrichia (Fig. 2.5c). The hairs on the femur are similar to those on the tibia (Fig. 2.6a); the longer hairs also have a double row of microtrichia (Fig. 2.6b). However the smaller hairs on the femur (Fig. 2.6c) have a double row of microtrichia, whereas four rows are present on the tibia.

The mouthparts of *Coloburiscus* are held beneath the head, the labial and maxillary palps being particularly prominent (Fig. 2.7). The setae and spines on the labrum, mandibles, labial palps and maxillary palps point towards the mouth. At the base of the maxillary palps are the maxillary gills (Fig. 2.7), which may be respiratory in function (Wisely, 1961). The labial and maxillary palps are scoop-like (Fig. 2.8). Food probably collects among the setae and on the large flat area of the galealacinia.

The labrum has prolific hairs directed towards the mouth cavity (Fig. 2.9a). These hairs have no microtrichia associated with them. The ventral view also shows short hairs on its underside (Fig. 2.9b). *Coloburiscus* nymphs appear to wipe food particles collected by the hairs on the legs onto the labrum. The labial and maxillary palps are then used to remove food from the labrum. The labrum therefore, acts like a "comb", removing food particles from the hairs on the legs.

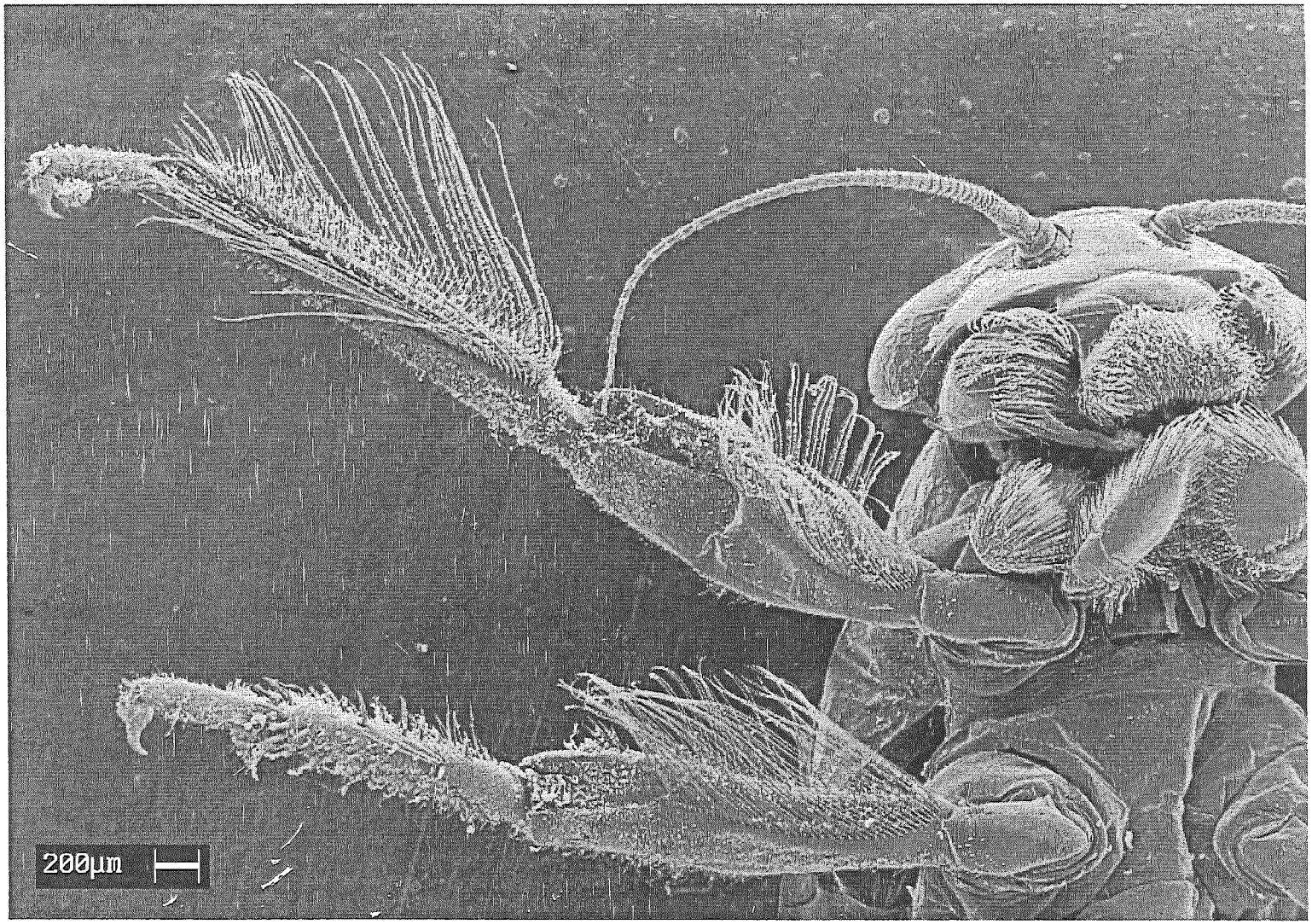


Figure 2.4: Mouthparts and the right pro- and mesothoracic legs of *Coloburiscus humeralis* (ventral view)

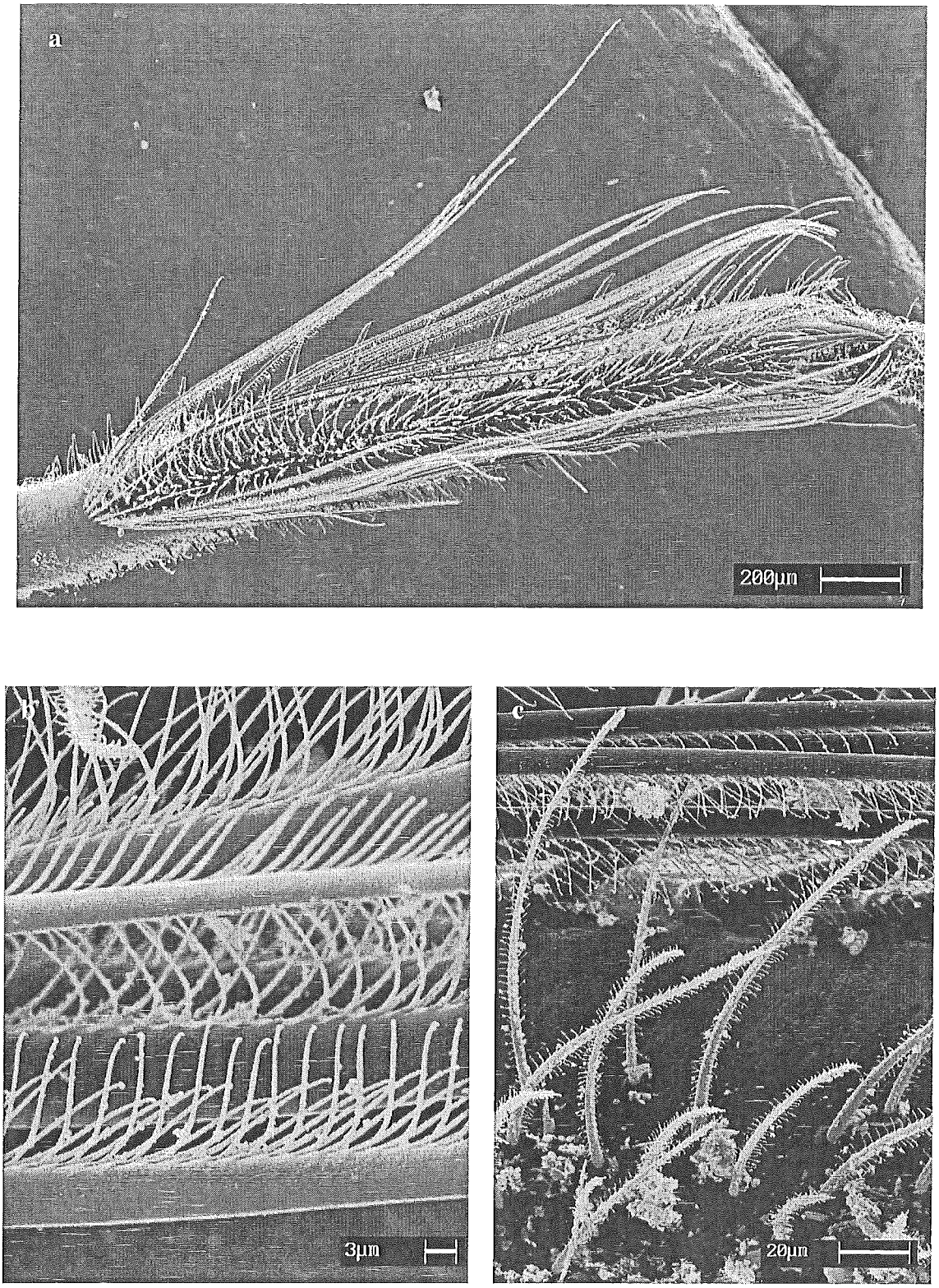
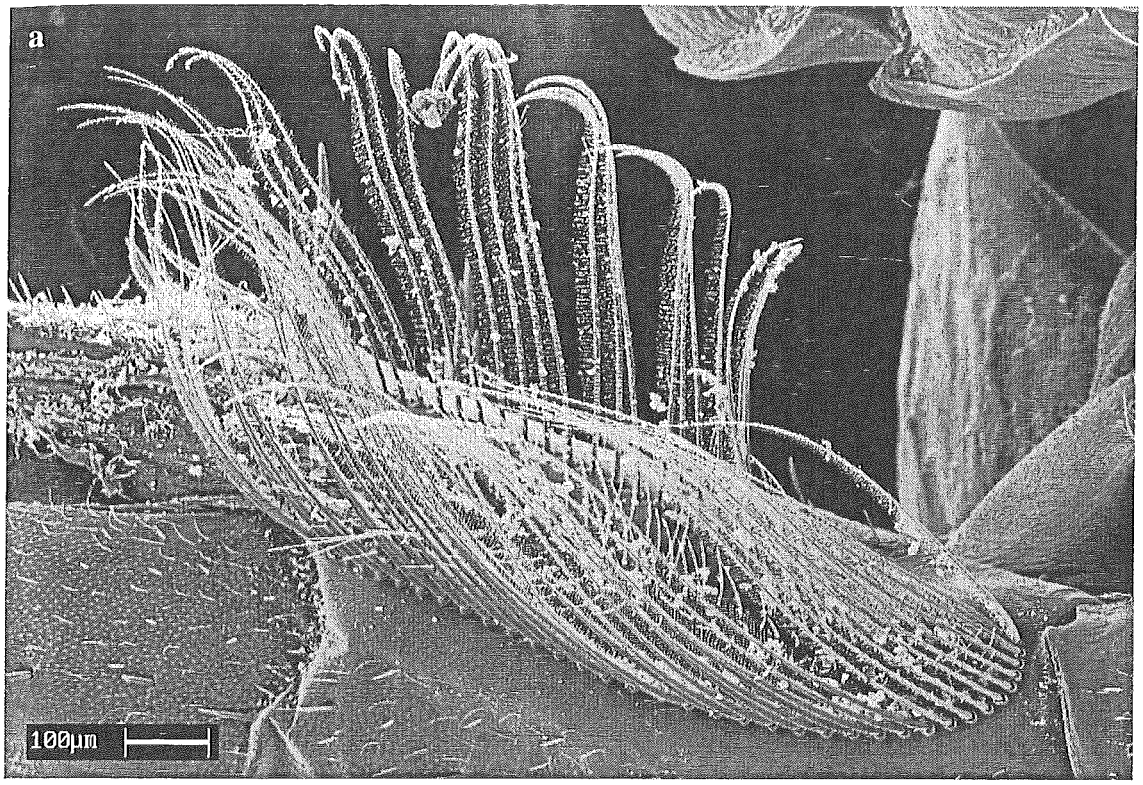


Figure 2.5: Hairs associated with the tibia of the foreleg of a *Coloburiscus humeralis* nymph (a). The longer hairs (b) have a double row of microtrichia, and the smaller hairs (c) have four rows of microtrichia.



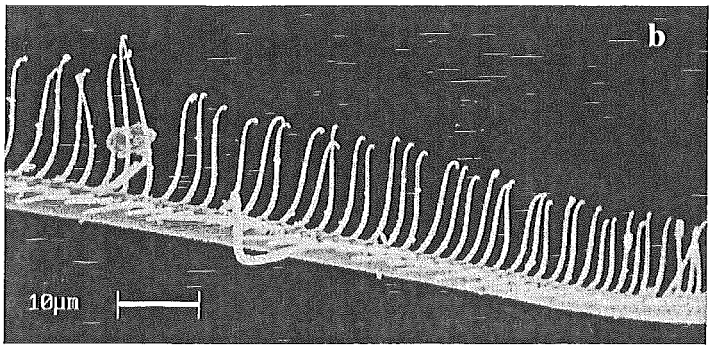
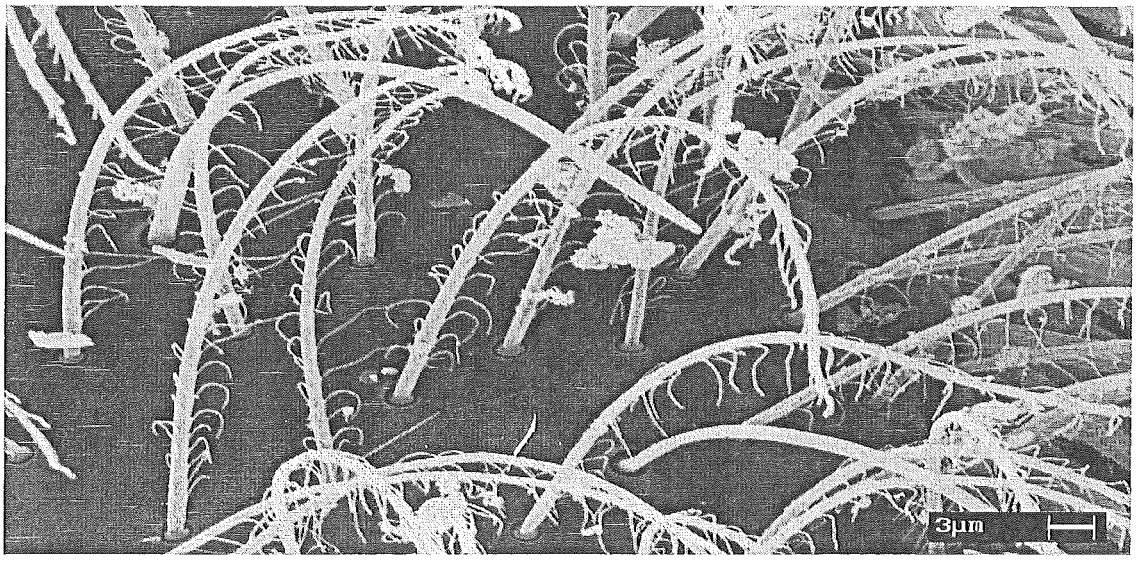


Figure 2.6: Hairs associated with the femur of *Coloburiscus humeralis* (a). The hairs are similar to those on the tibia, except the two types of hairs have a double row of microtrichia (b and c).



c

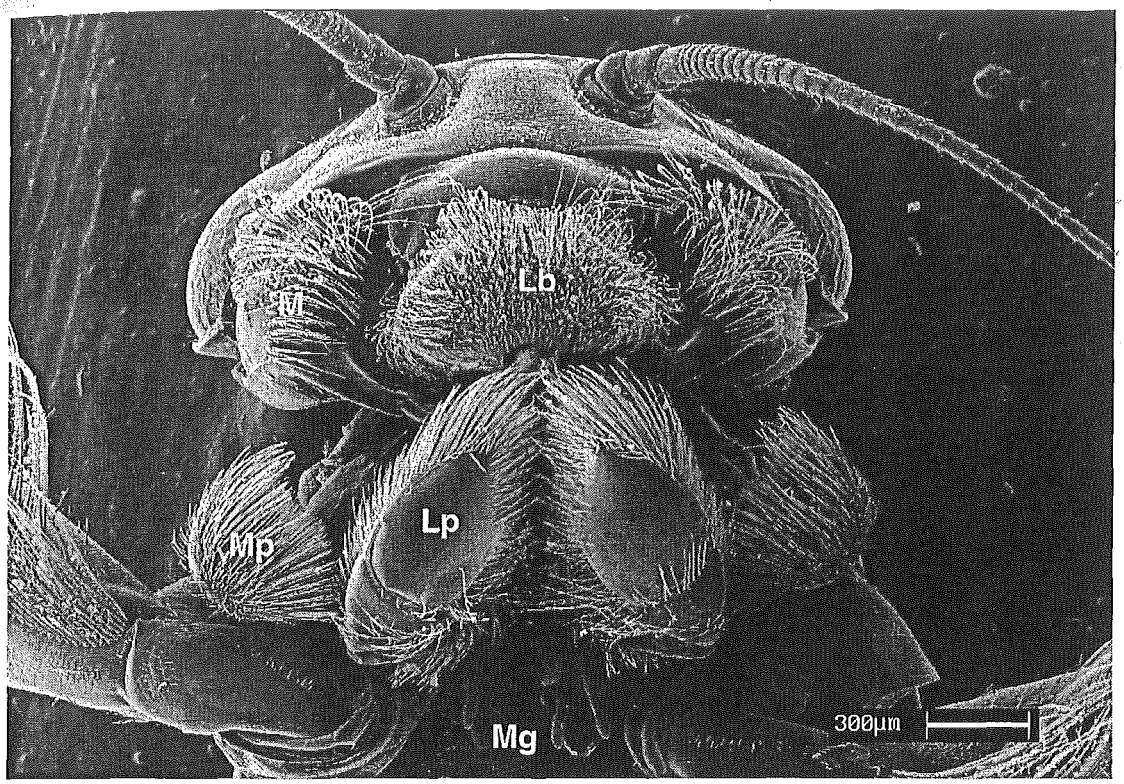


Figure 2.7: Mouthparts of *Coloburiscus humeralis* in ventral view. M = Mandible; Lb = Labrum; Lp = Labial Palp; Mp = Maxillary Palp and Mg = Maxillary Gills.

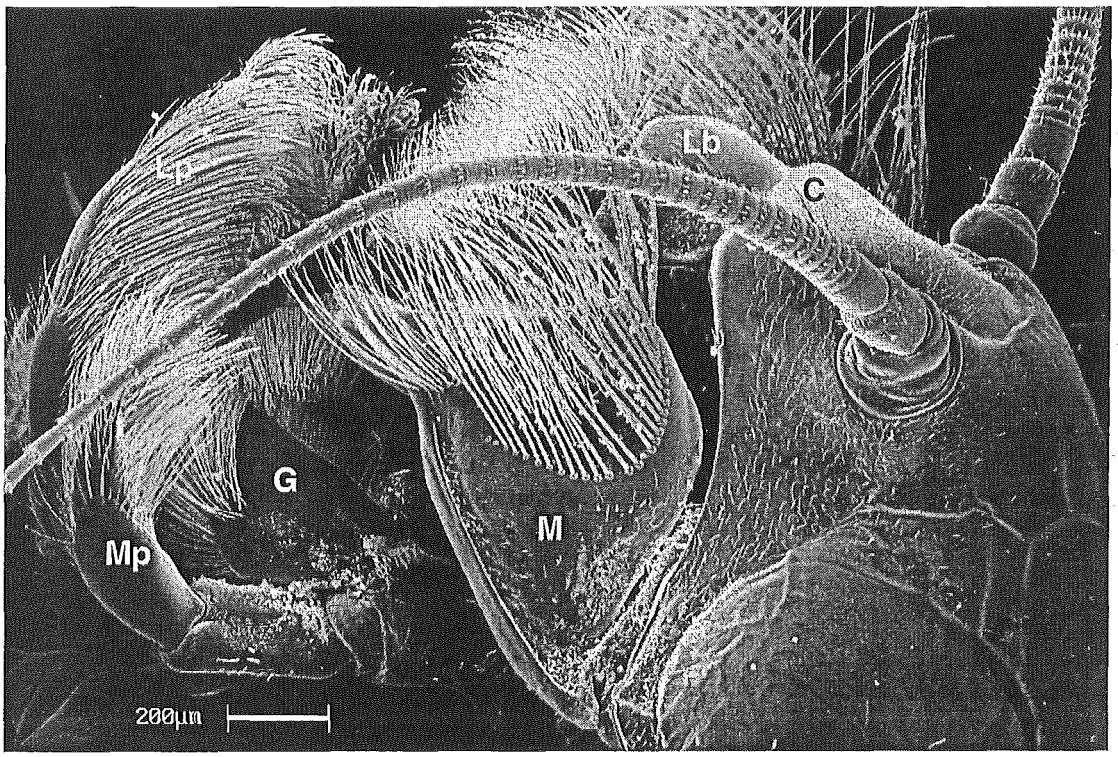


Figure 2.8: The large mandibles (M), labial palps (Lp) and maxillary palps (Mp) are prominent features of the mouthparts of *Coloburiscus humeralis*. The side view shows the scoop-like galealacinia (G) where food particles probably collect. Lb = labrum C = clypeus.

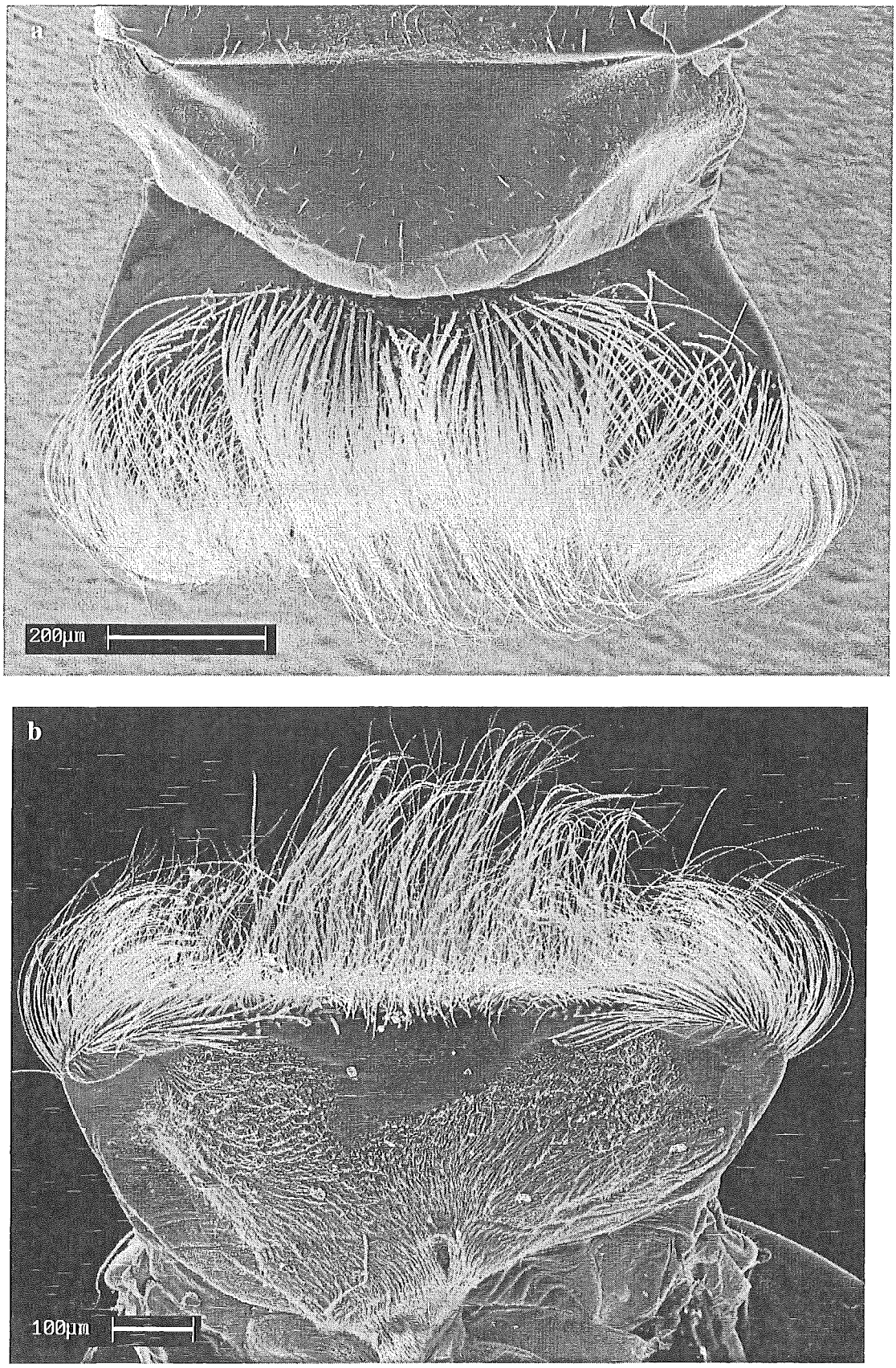


Figure 2.9: Labrum of *Coloburiscus humeralis*, (a) dorsal view (b) ventral view. Note: hairs do not have microtrichia and are all oriented towards the mouth cavity.

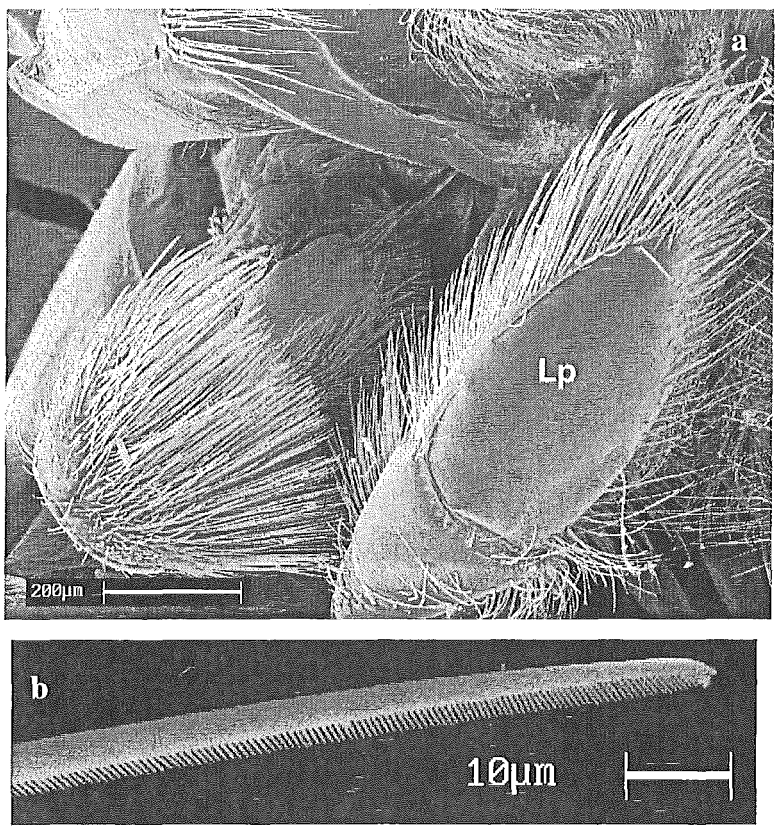


Figure 2.10: *In situ* view of labial palp (Lp) of *Coloburiscus humeralis* (a) and comb-like hair associated with the palp (b)

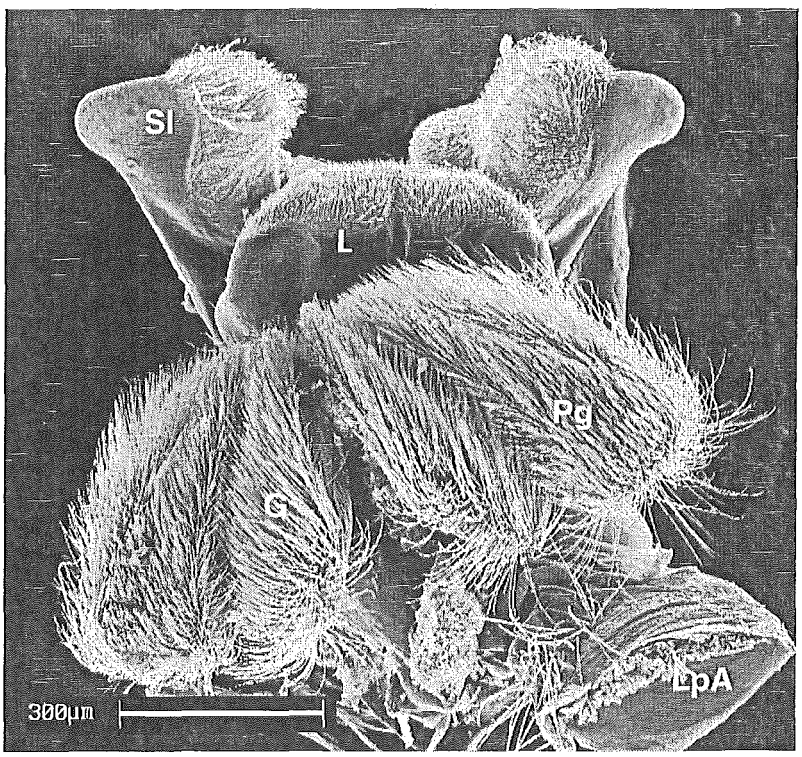


Figure 2.11: Ventral view of hypopharynx and labium of *Coloburiscus humeralis*. Sl = superlingua; L = lingua; Pg = paraglossa; G = glossa; LpA = attachment site of labial palp

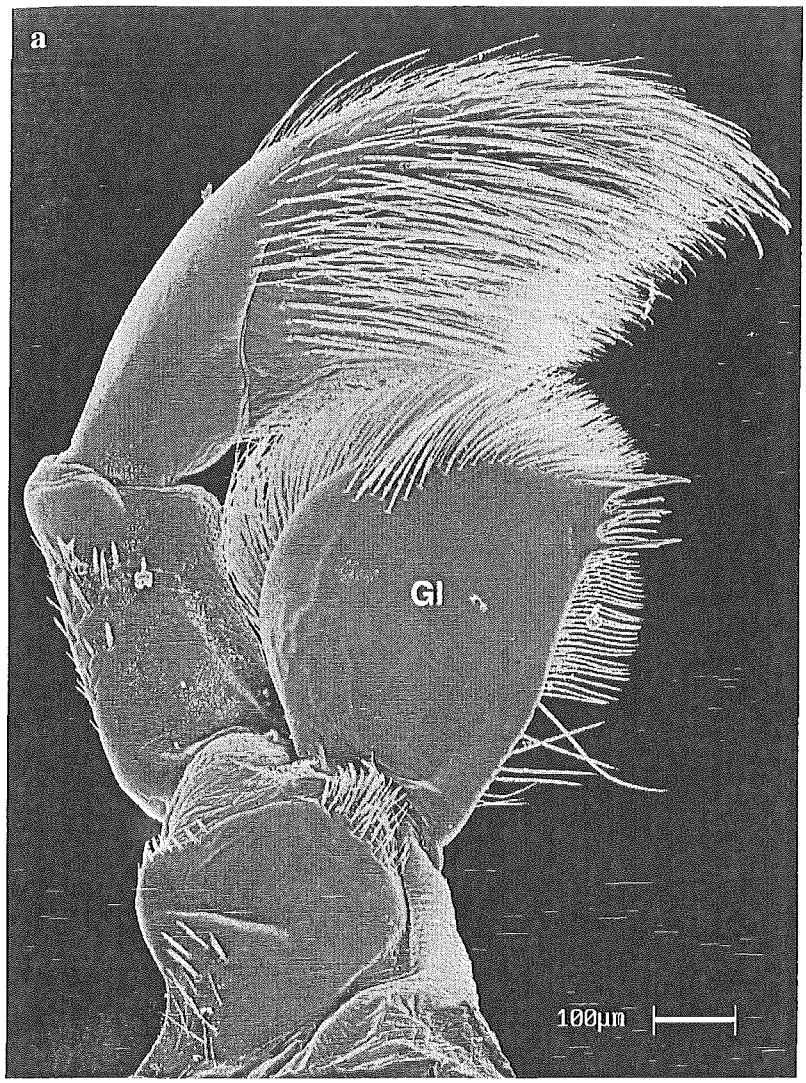
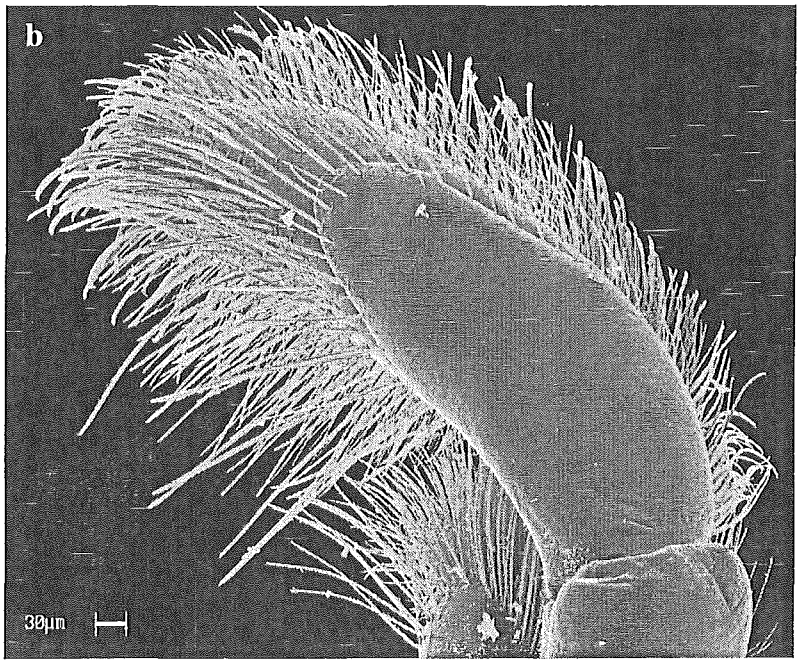
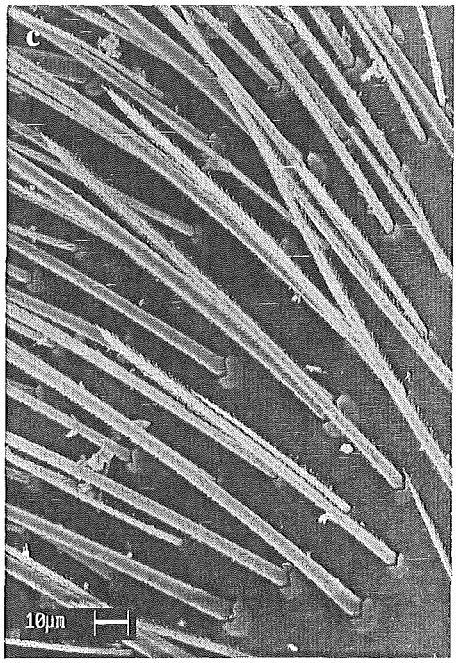


Figure 2.12: Ventral view of right maxillary palp of *Coloburiscus humeralis* (a) and hairs associated with tip of palp (b and c). Gl = galealacinia.





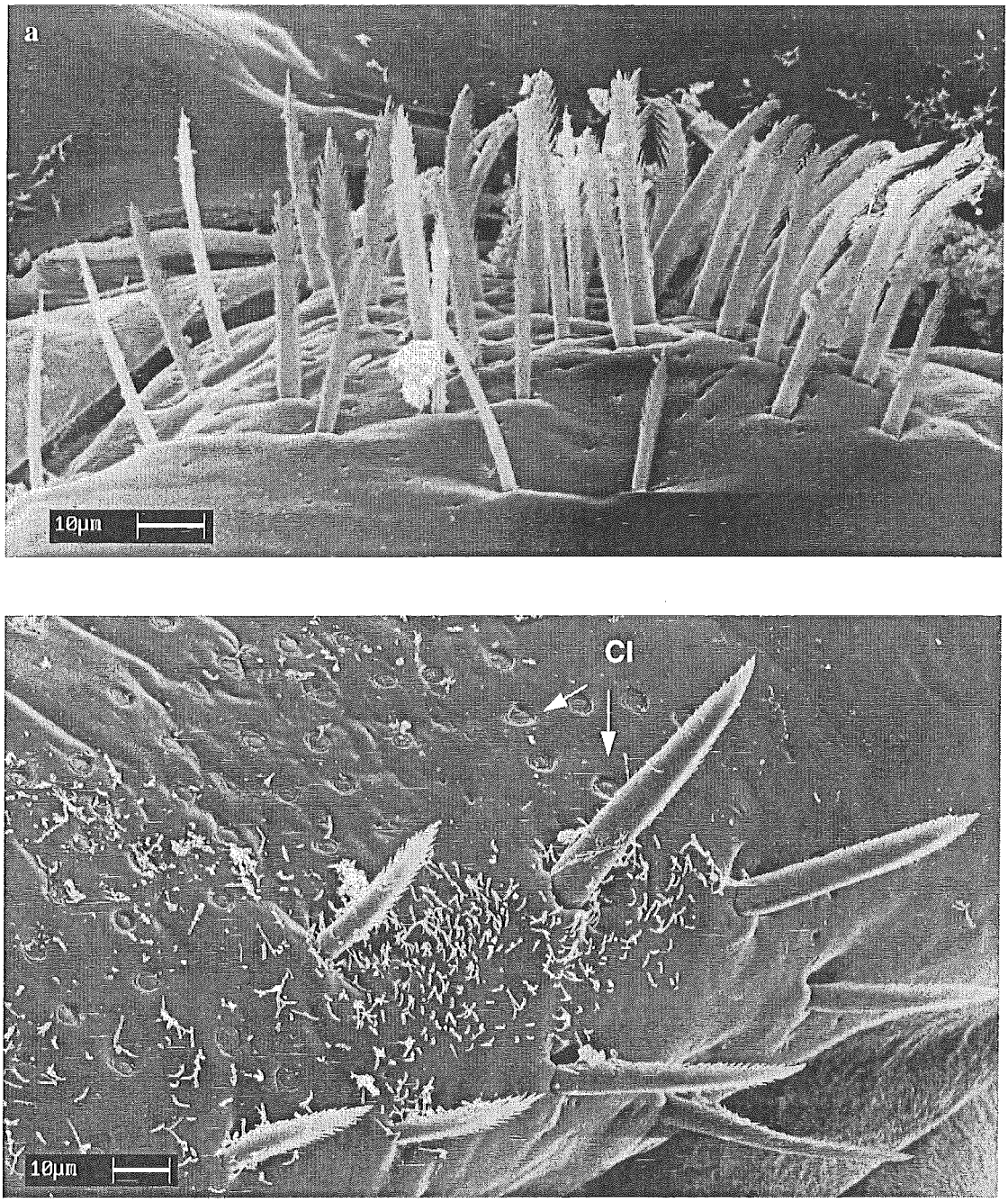


Figure 2.13: "Feathery" spines at base of galealacinia on maxillary palp (a) and on middle section of maxillary palp (b) of *Coloburiscus humeralis*. Associated with spines on the middle section are chloride cells (Cl).

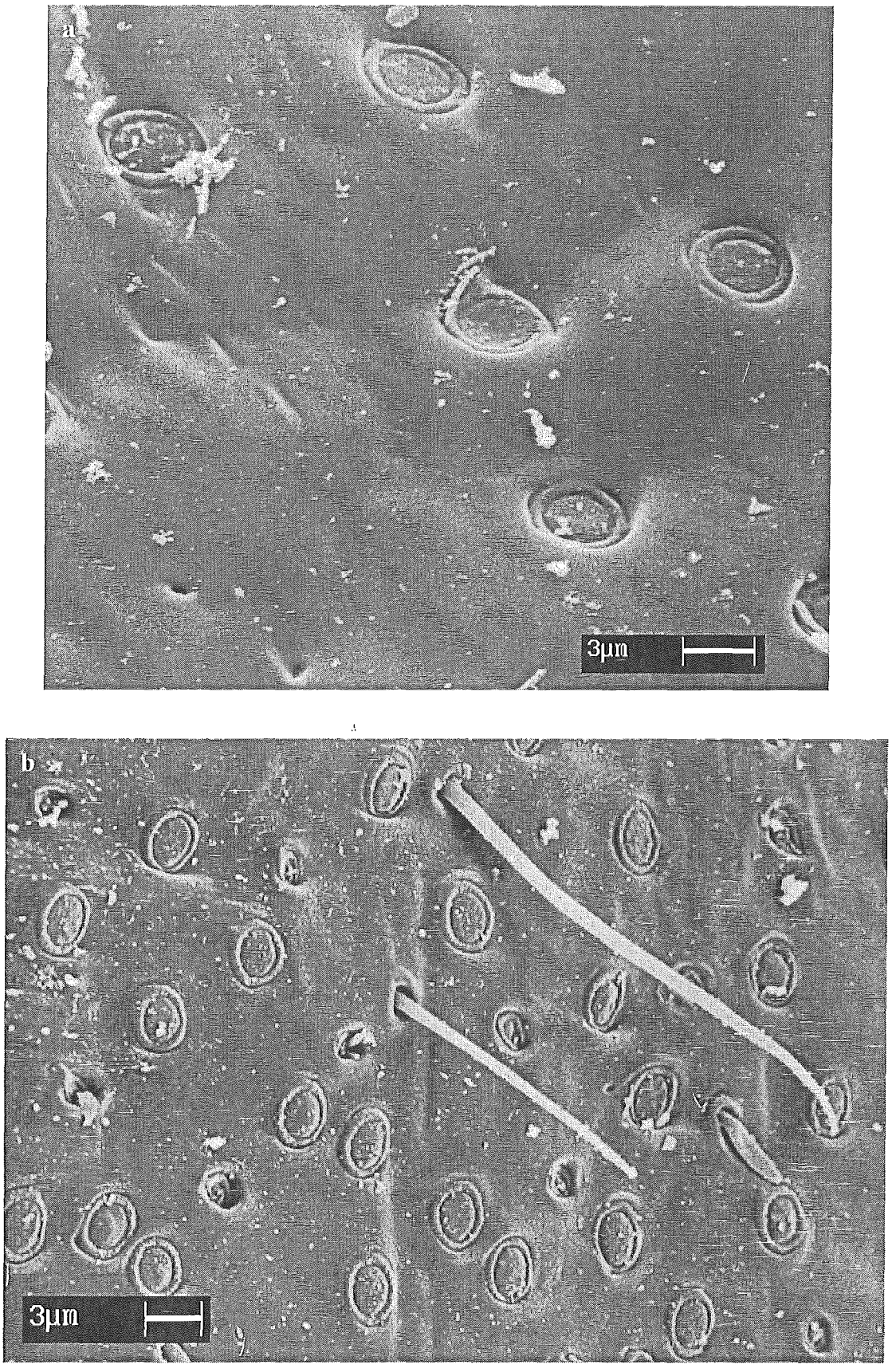


Figure 2.14: Chloride cells on maxillary palp (a) and abdomen (b) of *Coloburiscus humeralis*.

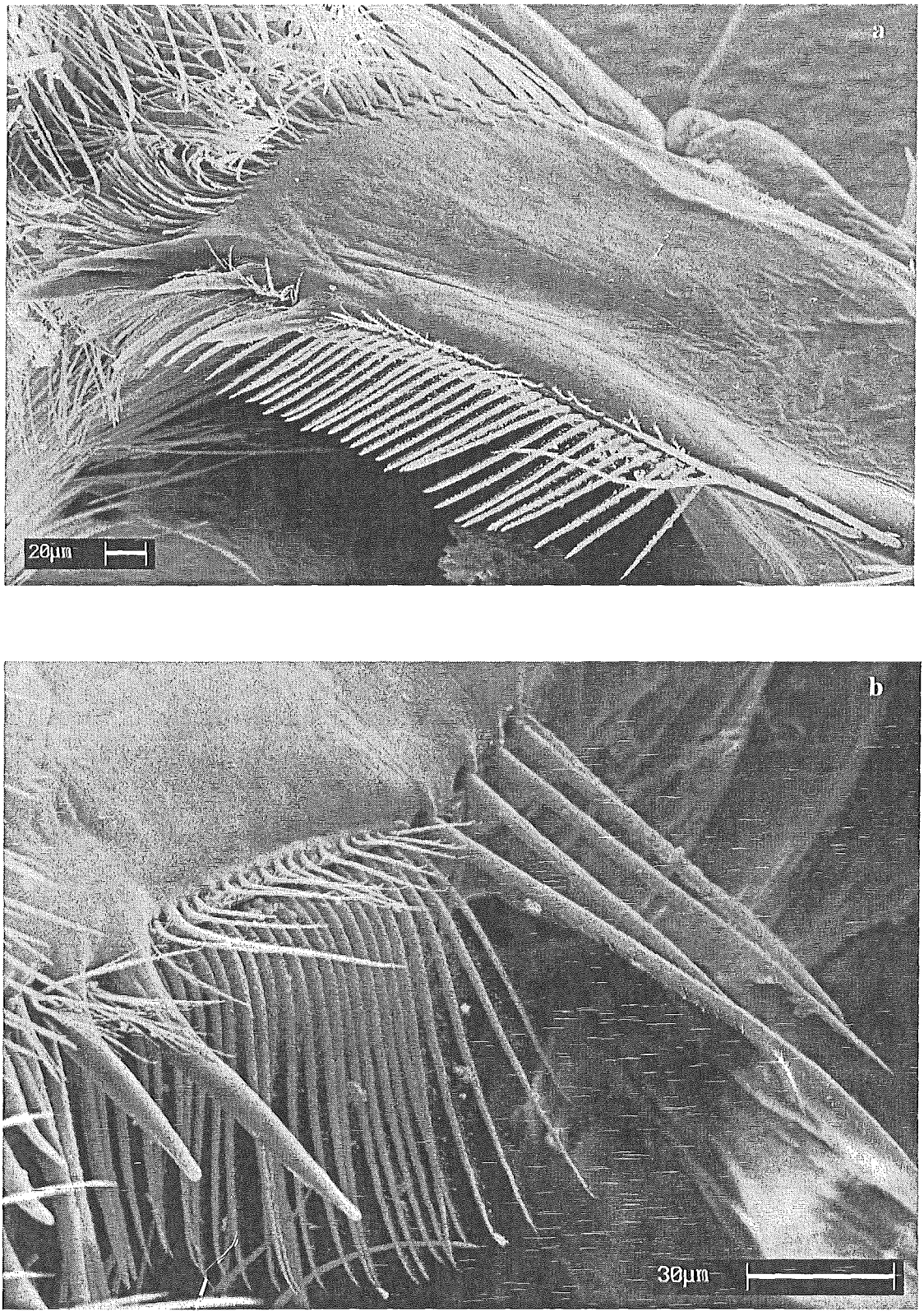
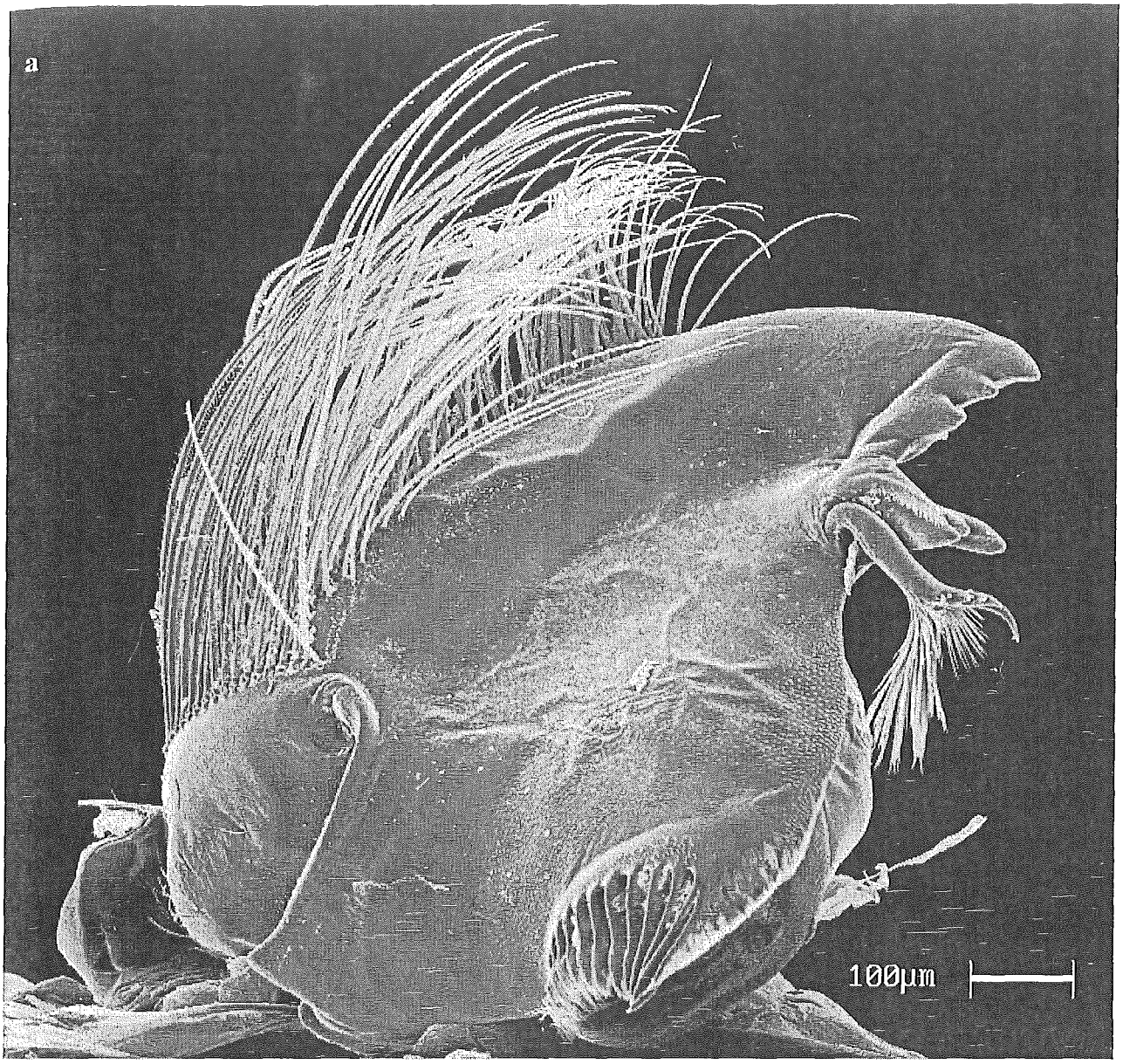
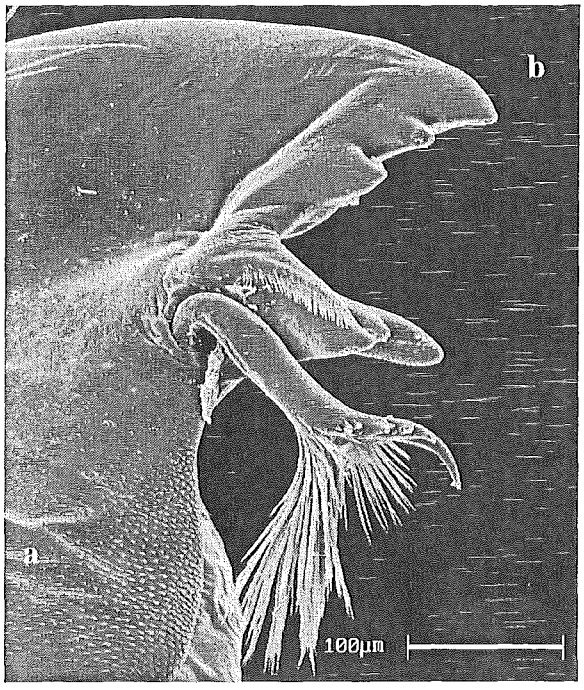


Figure 2.15: Galealacinia of *Coloburiscus humeralis* (a) and a closer view which shows the microtrichia associated with the hairs and spines on the galealacinia (b).





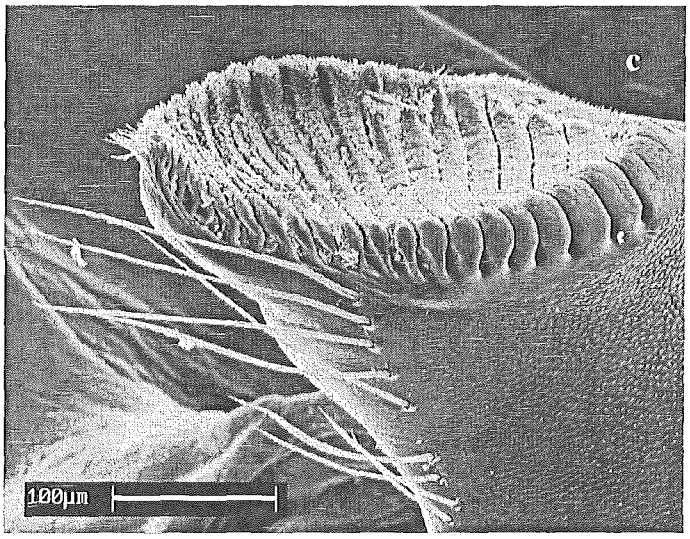


Figure 2.16: Right mandible of *Coloburiscus humeralis* nymph, (a) whole mandible (b) denticles (c) molar.

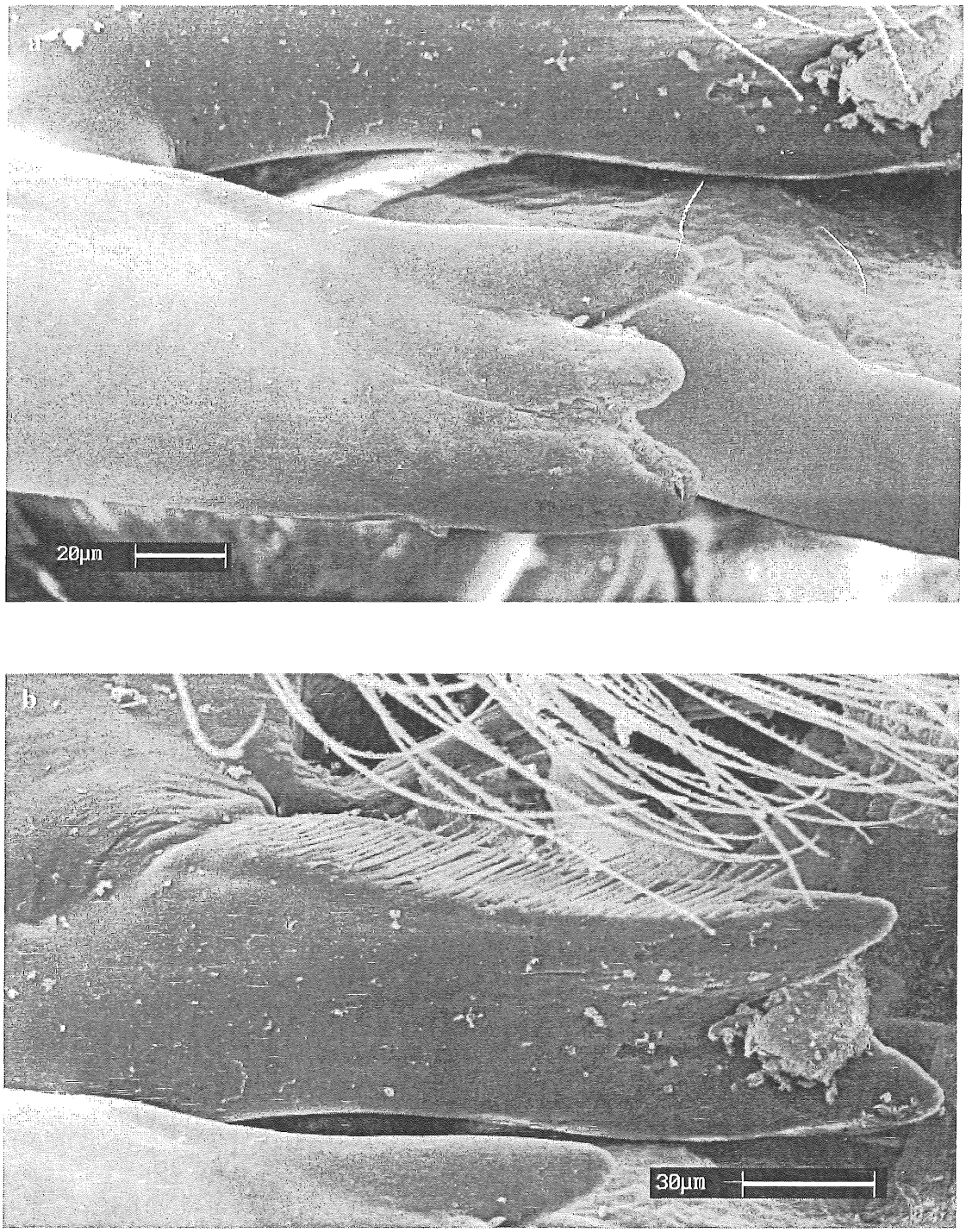


Figure 2.17: First (a) and second (b) denticles of the right mandible. The second set of denticles has comb-like hairs on the dorsal surface. Note: detritus caught between teeth.

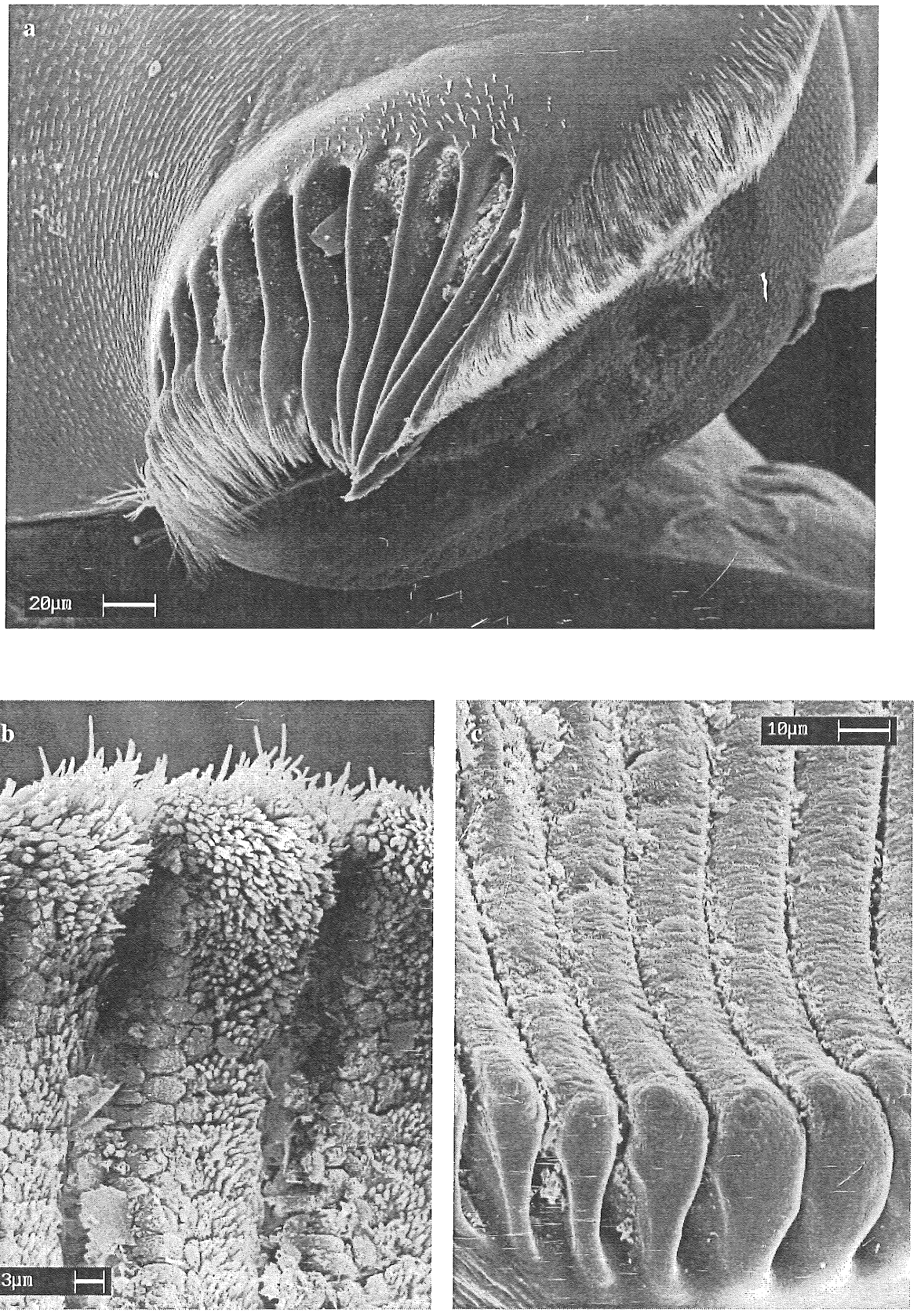


Figure 2.18: Molar of right mandible of *Coloburiscus humeralis* nymph (a), brush-like section of molar (b) and "chunky" grinding surface of molar (c).

Labium and Hypopharynx

The labium consists of the glossae, paraglossae and labial palps. The labial palps are similar to the maxillary palps (Fig. 2.10 a) and are situated below them. Their hairs are comb-like with a single row of microtrichia (Fig. 2.10 b), and are directed towards the mouth cavity. The paraglossae and glossae are extremely hairy (Fig. 2.11). These hairs have microtrichia similar to those of the maxillary palps. Small fine hairs are present on the superlingua, whereas the lingua has small short hairs (Fig. 2.11).

Maxillae

The maxillae each consist of a maxillary palp and a galealacinia (Fig. 2.12 a). The dorsal surface of the maxilla is covered in a variety of setae and spines. The tip of the maxillary palp has long setae, beginning in a prominent line and continuing around the palp (Fig. 2.12 b). The setae are articulated at the base and have comb-like microtrichia (Fig. 2.12 c).

Short setae and spines occur on the stipes below the base of the galealacinia and on the first segment of the maxillary palp (Fig. 2.13 a). The spines on the stipes are similar to those on lateral abdominal tergites of an oligoneuriid mayfly, *Elassoneuria* sp., where they are referred to as feathers (Agnew, 1980). In that species and *Coloburiscus humeralis* each spine originates from a single point, but in *Coloburiscus* the spines are grouped together. The cuticle around these spines also has small pore-like openings. The spines on the first segment of the maxillary palp are also "feathery" (Fig. 2.13 b) and associated with them are circular chloride cells (Fig. 2.14 a). Chloride cells are typically found in areas experiencing high current flows where they function as osmoregulatory structures (Stewart, 1993). For example, they are on maxillary palps and the abdominal gills of *Coloburiscus* nymphs (Fig. 2.14 b).

The galealacinia also has a variety of setae and spines associated with it (Fig. 2.15 a), and large numbers of stiff hairs run around its outer edges. The setae along the lateral edges of the galealacinia are in two rows with large spines at the anterior

end (Fig. 2.15 b). The setae on the anterior edge of the galealacinia are articulated and have microtrichia.

The hairs on the mouthparts of *Coloburiscus* nymphs are directed towards the mouth, and aids in the passage of particles to the other feeding structures. When the nymphs are feeding they part the labial and maxillary palps and wipe the food particles collected from the current onto the labrum and hypopharynx. It is probable that particles are then pushed further into the mouth cavity by movements of the labial and maxillary palps and especially the galealacinia. The labial palps may also protect the preoral cavity from the current.

Mandibles

The dorsal surfaces of the mandibles are equipped with long hairs with microtrichia (Fig. 2.7) and these hairs are oriented towards the mouth cavity. The mandibles have two sets of denticles or canines and a large molar (Fig. 2.16 a). Both sets of denticles have three teeth and the second set have comb-like hairs (Fig 2.16 b; Fig 2.17 a, b). The molar has a slightly concave surface (Fig. 2.16 c) with rows of brush-like hairs on the outer surface (Fig. 2.18 b) and solid grinding structures on the inner surface (Fig. 2.18 c). Food particles collect between the rows of hairs (Fig. 2.18 a)

The denticles on the mandibles are most likely used to grasp large food particles, such as leaf fragments, ready for the molars to grind. The outer molar region, with its rows of closely packed hairs, is probably used to remove excess water from among the filtered food particles as in some other mayfly species (e.g. *Chloeon dipterum*: Brown, 1961; *Hexagenia* spp.: Flannagan and Marshall, 1980; *Palingenia longicauda*: Landolt et al., 1995). The function of the bristled prostheca on the mandibles is not clear (Flannagan and Marshall, 1980).

2.3.3 Conclusion

Observations of feeding behaviour and mouthpart morphology confirm that *Coloburiscus humeralis* is a passive filter feeder. Hairs on the tibiae and femora of the legs are covered with microtrichia and act as nets to capture food particles. The

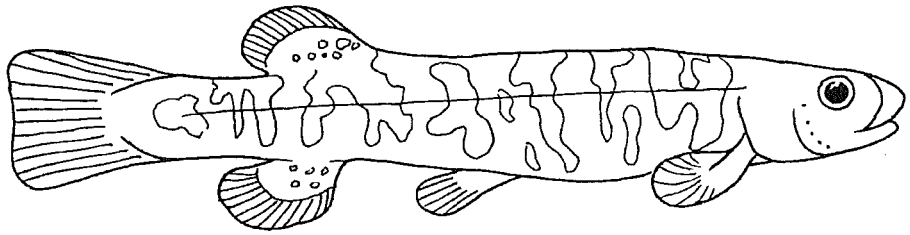
microtrichia associated with these hairs and also those on the mouthparts may increase the surface area of the hairs, enabling greater rates of particle capture. The presence of bipectinate and pectinate (pectinate setae are setae which have one row of equal or unequal microtrichia; bipectinate setae have two rows of microtrichia) microtrichia is consistent with the classification of *Coloburiscus* as a passive filter feeder (Palmer, 1998). Observations of *Coloburiscus* also support this, since nymphs did not actively move legs or mouthparts to capture food particles. Other species of filter feeding mayflies (e.g. Oligoneuriidae) also have long hairs on the legs specialized to function as a net (Agnew, 1980; Elpers and Tomka, 1995).

Oligoneuriid nymphs like Coloburiscidae, characteristically have filter brushes on the tibiae and femora of the legs (Agnew, 1980). As in *Coloburiscus,* the main function of these may be to sieve food particles from the water column. However, they may also be used to rake the surface of the substrate (Agnew, 1980) thereby bringing fine particles into suspension. Such behaviour was not observed in *Coloburiscus* but it is possible that it occurs in a natural stream environment.

The most important adaptation of filter feeding insects for suspension feeding is the presence of hairs with microtrichia. *Coloburiscus* has many kinds of microtrichia associated with hairs on the mouthparts and legs, and together they enable nymphs to collect food particles of various sizes from the current. Other passive filter feeders such as hydropsychid caddisflies use nets attached to the substrate; *Coloburiscus* nymphs effectively have a portable version of these.

Seston is a relatively low energy food, and passive filter feeders, such as *Coloburiscus*, rely heavily on stream current to transport large numbers of particles to them. At high velocities, there may be greater food supply but there may also be increased risks of being swept away or of filter-feeding structures being damaged or ineffective. Therefore, habitat selection by *Coloburiscus* nymphs may involve a trade-off between sites with a good supply of seston and areas where velocity is slow enough for the nymphs to persist and filter efficiently.

CHAPTER



3

CHAPTER THREE

Feeding Behaviour of *Coloburiscus humeralis*

3.1 INTRODUCTION

Two major factors influencing the life history and behaviour of stream invertebrates are food supply and predators (see Hynes, 1970; Allan, 1995; Winterbourn, 2000). These two factors are often linked via a trade-off between foraging and predator avoidance (see Kerfoot and Sih, 1987: Malmqvist and Sackmann, 1996). For example, *Deleatidium,* a grazing mayfly had fuller guts at night when predatory trout were present, and fuller guts during the day in streams with native galaxiids (McIntosh and Townsend, 1995). Top-down control by predatory fish has been documented in many grazing invertebrate species (Culp and Scrimgeour, 1993; Peckarsky et al, 1993; Cowan and Peckarsky, 1994; McIntosh and Townsend, 1996; Huhta et al, 1999) and quantifying the effect of food supply on grazing invertebrates is comparatively easy. Logistically, measurements of particulate organic matter are much harder (Gee, 1991) and there have been few observations of the effect of predation on filter feeders.

In the previous chapter various morphological adaptations used by *Coloburiscus humeralis* for feeding were examined. The purpose of this chapter is to investigate the effect of predatory fish and abiotic factors on the feeding of *Coloburiscus humeralis*. Morphological adaptations in *Coloburiscus* for fine particle filter feeding include specialized hairs on the mouthparts and legs (Chapter Two). Behavioural adaptations may also be important for obtaining a food. *Coloburiscus* is

most commonly found under rocks and stones (Wisely, 1961), however, habitats of other filter feeding species (e.g. Simuliidae larvae) include the tops of rocks where the stream current provides a continuous food supply. Behavioural feeding adaptations may allow *Coloburiscus* nymphs to live and feed in the conditions found under rocks, but their need to filter requires that they must be in relatively fast currents.

Filter feeding species may obtain food particles actively or passively from the stream current. Some species may be discriminatory but the majority are probably not (e.g. *Coloburiscoides*: Campbell, 1985). Quantity and quality of seston have been assessed in relation to the secondary production of filter feeding species (Wallace and Merritt, 1980; Merritt et al, 1982; Cummins and Klug, 1979). For example, Simuliidae and hydropsychid larvae are often associated with lake outlets (Harding, 1997; McCreadie and Robertson, 1998) where seston quality may be high (Wallace and Merritt, 1980). However, in New Zealand two species of *Aoteapsyche* that consumed seston differing in quality had similar growth rates (Harding, 1997), perhaps because seston quantity was more important than its quality. In contrast, Merritt et al (1982) found that quantity of seston did not limit production of filter feeding species (blackfly larvae), suggesting that the effect of seston quality and quantity on secondary production is quite variable and depended on the species concerned.

Position in the water column is extremely important for obtaining food in filter feeding species. Simuliid and hydropsychid larvae generally are associated with the tops of rocks (Harding, 1997, Winterbourn et al., 2000). Simuliid larvae use cephalic fans to actively filter fine particles from the current (Ross and Craig, 1980; Craig and Chance, 1982), whereas *Aoteapsyche* (a New Zealand hydropsychid) passively collect food particles using silk nets in crevices between rocks (Harding, 1997). Therefore, if nymphs avoid predators by moving from areas of high food availability there are likely to be pronounced effects on the growth and production of that species. For example, the mayfly *Oligoneuriella marichuae*, is associated with filamentous algae in cobbled reaches of Spanish streams (Alba - Tercedor, 1990). This habitat may be used as a refuge from predation, but it may also act as a "food filter" for smaller nymphs whose feeding apparatus is not fully developed (Alba – Tercedor, 1990). The filamentous algae trap FPOM, which may be collected by small *Oligoneuriella*

nymphs. Therefore, selection of suitable habitats may be based on low risks of predation but also high rates of food supply.

Grazing invertebrate species may avoid drift or drift in response to predatory fish to escape predation. For some invertebrates, predators may induce prey to find refuge under rocks or some other form of substrate (Tikkanen, Muotka and Huhta, 1994). Larval blackflies reduce the risk of predation from predatory invertebrates by inhabiting areas of fast current velocity (Malmqvist and Sackmann, 1996) where predatory caddisfly and stonefly species are less successful at capturing them. However, feeding by *Simulium* larvae is also reduced in faster currents (Malmqvist and Sackmann, 1996). Therefore, changes in microhabitat to avoid predation may be costly.

Many studies that have examined the effects of predators on prey species have highlighted their lethal effects, i.e., consumption of the prey species. However, sub-lethal effects, including anti-predator defences and changes in behaviour, are also important (Johansson and Samuelson, 1994; Peckarsky, 1996; Straile and Halbich, 2000; Dahl and Peckarsky, 2002). Predator avoidance behaviour has been shown to lower fecundity in mayflies by affecting growth rates (Peckarsky et al., 1993) and by altering feeding behaviour (Poff, DeCino and Ward, 1991; McIntosh and Townsend, 1994; McIntosh and Townsend, 1995). The response of grazing invertebrates to predation by fish and invertebrates has been examined widely (Poff, DeCino and Ward, 1991; Tikkanen, Muotka and Huhta, 1994; Miyasaka and Nakano, 2001). However, the effect of predators on other functional feeding groups is not well known. In many cases, predatory fish have been observed to have little effect on non-algal based food webs (e.g. detritus-based streams: Reice, 1991). Therefore, it is possible that invertebrate predators may have a more significant effect because they occupy the same microhabitat as their prey (Kohler, 1992).

Abiotic factors also may affect the food supply and alter the impact of predation on filter feeding species. For example, *Aoteapsyche colonica* had a positive association with current velocity and substrate size in Grasmere Stream at Cass and Blue Duck Stream in Kaikoura (Winterbourn and Harding, 1993). Current velocity determines the food supply of filter feeders, and larger substrates may provide a stable habitat for feeding (Hynes, 1970). Two species of *Aoteapsyche* were found to co-

exist because of differences in microhabitat preferences (Harding, 1997). One species was able to persist on the tops of rocks, while the other preferred the sides or undersides of rocks (Harding, 1997). Abiotic factors therefore may reduce competition between these two species. Current velocity may also mediate the effect of predatory invertebrates on filter feeding species (Malmqvist and Sackmann, 1996). Higher velocities reduced the effectiveness of predation by stonefly and caddisfly predators on *Simulium* larvae in an experimental flume (Malmqvist and Sackmann, 1996). Therefore, abiotic factors may alter the impact of biotic variables, such as predation and food availability.

The impact of predation by fish on the feeding behaviour of *Coloburiscus* may not be significant, as nymphs inhabit the undersides of rocks. However, as discussed in Chapter One, filter feeding invertebrates rely to some extent on other species to break CPOM down into FPOM. Transport of FPOM in streams is also dependent on the movement of invertebrates and possibly fish. Therefore, it is possible that predatory fish will affect the feeding behaviour of *Coloburiscus* indirectly via their impact on other invertebrate species. Predatory fish may reduce the processing abilities of other invertebrate species directly via predation or indirectly by reducing movement. Reduction in movement of invertebrate species may also decrease the rate of food supply to *Coloburiscus* nymphs.

Gut fullness has been used in many studies to examine the effect of predatory fish on the feeding activity of their potential prey species (Culp and Scrimgeour, 1993; Peckarsky et al, 1993; McIntosh and Townsend, 1995; Huhta et al, 1999). In this chapter, gut fullness was used to determine the effect of native and introduced fish predators on the feeding behaviour of *Coloburiscus humeralis*. It is unlikely that predatory fish will affect the feeding behaviour of *Coloburiscus* nymphs directly but the indirect of predators on other invertebrate species may affect the food supply of *Coloburiscus*.

3.2 METHODS

3.2.1 Study Sites

Sampling sites were selected from three regions in the South Island of New Zealand. Sites at Cass (Southern Alps) and Hanmer (120 km north west of Christchurch) are located in the "high country ecoregion" and sites on Banks Peninsula are within the "Banks Peninsula ecoregion" (Harding and Winterbourn, 1997).

The high country ecoregion encompasses the hill country and foothills east of the Southern Alps and west of the Canterbury plains (Harding, 1994). Elevation of sampling sites within this ecoregion ranged from 280 to 1000 m. The dominant vegetation in the high country ecoregion is tussock (*Chionochloa* spp.), with stands of beech forest (*Nothofagus* spp.) present in the Cass-Craigieburn region and near Hanmer. Riparian vegetation at Cass was dominated by either beech forest (Fig. 3.1) or tussock (Fig. 3.2), with some scrub (Fig 3.3) including broom (*Cytisus scoparius*) and gorse (*Ulex europaeus*). Introduced trees mostly *Pinus radiata* (Fig. 3.4) were present in the Hanmer area. Substrate at the Cass and Hanmer sites was typically dominated by small to large cobbles (6 – 25 cm).

The vegetation of the Banks Peninsula ecoregion is strongly influenced by the region's volcanic history. The fertile soils and high rainfall (p 72, Knox, 1969; Harding, 1994) are reflected in the originally diverse and lush native vegetation present on Banks Peninsula. However, pasture grasses and tussock (*Poa* and *Festuca* spp.) now dominate, where human impact has been substantial. There are also large areas of native scrub including bracken (*Pteridium esculentum*), mahoe (*Melicytus ramiflorus*), fucshia (*Fucshia excorticata*), wineberry (*Aristotelia serrata*) and *Hebe* (Harding, 1994) in regenerating areas.

The four streams sampled on Banks Peninsula generally had a higher density of vegetative cover than the high country streams (Fig. 3.5 and 3.6). Riparian vegetation included mahoe, *Coprosma*, fuchsia, peppertree (*Macropiper excelsum*) and wineberry. The streams were dominated by large cobbles and boulders (> 13 cm).

Figure 3.1: Beech (*Nothofagus* sp.) dominated riparian vegetation at Binser Saddle stream (left) and Camp Stream (right) near Cass. Binser Saddle stream contains *Coloburiscus* while Camp Stream does not.

Figure 3.2: Slovens Stream (left) and Coach Stream (right) near Cass are surrounded by *Chionochloa* spp. (tussock grassland).

Figure 3.3: A mix of gorse (*U. europaeus*), broom (*Cytisus scoparius*) and grasses dominate riparian vegetation at Mt White Hut Stream (left) and Manson Creek (right) near Cass.

Figure 3.4: Riparian vegetation at Hanmer sites is similar to that near Cass. "Tequila" Stream (left) is surrounded by tussock and grasses and Mt Isobel Stream (right) is bordered by a mix of native and introduced trees.

Figure 3.5: The riparian zones of streams on Banks Peninsula are dominated by broadleaf species, as at the densely shaded Kinloch Rd Stream.

Figure 3.6: Much of Okuti Valley Stream on Banks Peninsula is shaded by dense riparian vegetation but it is significantly lighter in areas where vegetation had been removed.

The twenty two study sites on first and second order streams were selected on the basis of their accessibility and the presence or absence of predatory fish. They were classified as: fishless (no trout or galaxiids), galaxiids only, trout only, or both trout and galaxiids present. Other fish species present were also recorded.

3.2.2 Sampling methods

Predatory fish densities were measured between 19 July and 12 September 2001 using a single quantitative pass of a 50 metre reach of each stream with an electric fishing machine at Hanmer and Banks Peninsula sites (McIntosh, 2000). Total length of each fish caught was measured to the nearest centimetre and then fish were released. Fish data for streams at Cass were collected by electrofishing by Angus McIntosh and Per Nyström (McIntosh, 2000). Total fish biomass, galaxiid biomass and trout biomass were determined for each stream.

Four streams were selected from each region based on the type of predatory fish present as indicated above. Sampling was undertaken in September 2001. Five samples were taken from each stream, once at night (approximately two hours after sunset) and once during the day (between 10 am and 3 pm) using a 0.0625 m^2 Surber sampler. Samples were placed in 10% formalin to preserve gut contents.

Velocity and depth were measured once at each sample site in a riffle section of the stream using an EDSC NIWAR Current Meter. Twenty litre water samples were taken night and day to estimate seston concentration at each stream. A plastic pipe (internal diameter = 5 cm) was placed at the surface in each stream prior to sampling and water was collected via this pipe into a plastic container. The pipe was used to avoid disturbing the stream bed and bringing FPOM into the water column. Water samples were filtered through glass fibre filters (Whatman Glass Microfibre Filters, pore size $\approx$ 1μm) using a Millipore vacuum system within four days of collection.

Five medium to large sized nymphs were selected from each sample for gut analysis. Head capsule and total body length (excluding cerci and telofilum) were measured, to the nearest 0.1mm, using a linear eyepiece micrometer at a magnification of 8 x. *Coloburiscus* nymphs were then dissected under a microscope

and the whole gut was removed. Gut contents were placed on pre-weighed filters and dried in an oven at 60°C overnight. Dried samples were weighed on a Cahn microbalance to the nearest 0.01mg.

A body length versus dry weight regression was constructed to allow gut fullness to be standardized in terms of body dry weight from length measurements. The body lengths of 65 nymphs were measured and nymphs were dried at 65 °C for 24 h. Each individual was then weighed to calculate the relationship between body length and weight. This relationship was used to determine gut content weight as a percentage of total body weight. Regression analysis showed that *Coloburiscus humeralis* body length was closely related to dry weight ($r^2 = 0.93$, $p < 0.05$) (Fig. 3.7).

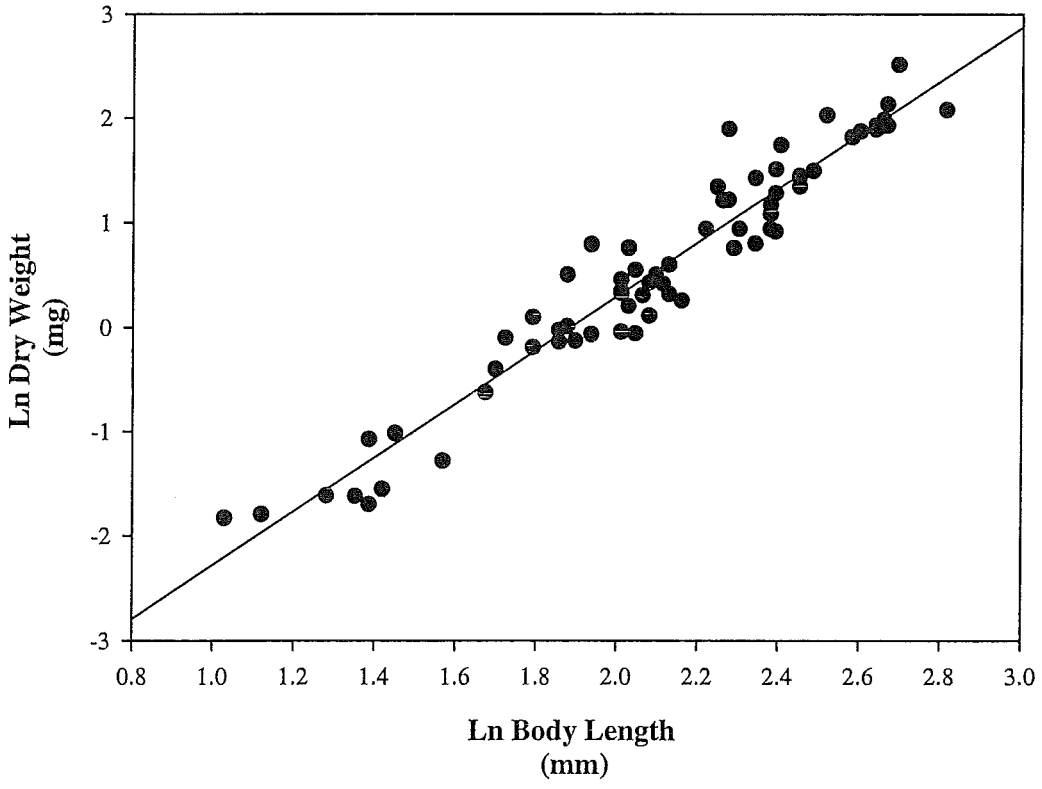


Figure 3.7: Relationship between body length (measured from head to end of abdomen) and dry weight of *Coloburiscus humeralis* (DW (mg) = $0.008L^{2.57}$) ($r^2 = 0.93$, $p < 0.05$).

Table 3.1: Predatory fish found in study streams at Cass, Hanmer and Banks Peninsula when electrofished. "+" indicates fish species present. Predatory fish treatments: NF – fishless; G – galaxiids only; T – trout only; T + G – trout and galaxiids present.

Region	Site	alpine galaxiid	Canterbury galaxiid	banded kokopu	koaro	upland bully	common bully	shortfin eel	longfin eel	brown trout	Chinook salmon
Cass*	NF										
	G		+			+			+		
	T								+	+	
	T + G	+	+						+		+
Banks Peninsula†	NF								+		
	G			+	+				+		
	T					+			+	+	
	T + G				+	+			+	+	
Hanmer†	NF										
	G		+			+		+	+		
	T									+	
	T + G		+			+	+		+	+	

* electrofishing data collected during 2000 by A. McIntosh and P. Nyström.

† electrofishing data collected between 19 July and 12 September 2001.

To examine the gut contents of *Coloburiscus* nymphs, ten medium to large sized nymphs were dissected under a stereo microscope and gut contents were removed. The gut contents were mounted on a slide in lactophenol PVA, and allowed to dry and clear for four days at room temperature. The composition and size range of particles in gut contents was examined microscopically at a magnification of 100 x. Photographs of slides were taken using a Zeiss Axioskop 2 MOT microscope.

3.2.3 Statistical Methods

To determine whether predatory fish had an effect on the weight of gut contents in *Coloburiscus humeralis* nymphs a two-way ANOVA was used. The four treatments: fishless, galaxiid only, trout only and both trout and galaxiid were replicated with samples from different regions. The data (mg of gut contents/ mg of dry body weight) were $\log_e (x + 1)$ transformed to homogenize variances. Gut contents of *Coloburiscus* nymphs at night and during the day were analysed separately. Region was initially included in the ANOVA as a blocking term but was subsequently removed from all analyses because it was not significant. Pearson correlation was used to examine the relationship between organic and inorganic component of seston, total seston and gut fullness of *Coloburiscus* nymphs.

3.3 RESULTS

The gut contents of *Coloburiscus humeralis* were dominated by amorphous detritus (FPOM), constituting approximately 54% of total gut contents (Fig. 3.8) (Fig. 3.9). Inorganic material consisting mainly of crystalline particles (most likely sand) was also important, making up 13% of the total gut contents (Fig. 3.8). Other food items detected, included large invertebrate mouthparts (Fig. 3.10 a and b), pollen grains and diatoms (Fig 3.10 c and d). The maximum linear dimension of food items was 650 μm (invertebrate mouthparts, Fig 3.10 a) and the smallest were less 10 μm (diatoms, Fig 3.10 d).

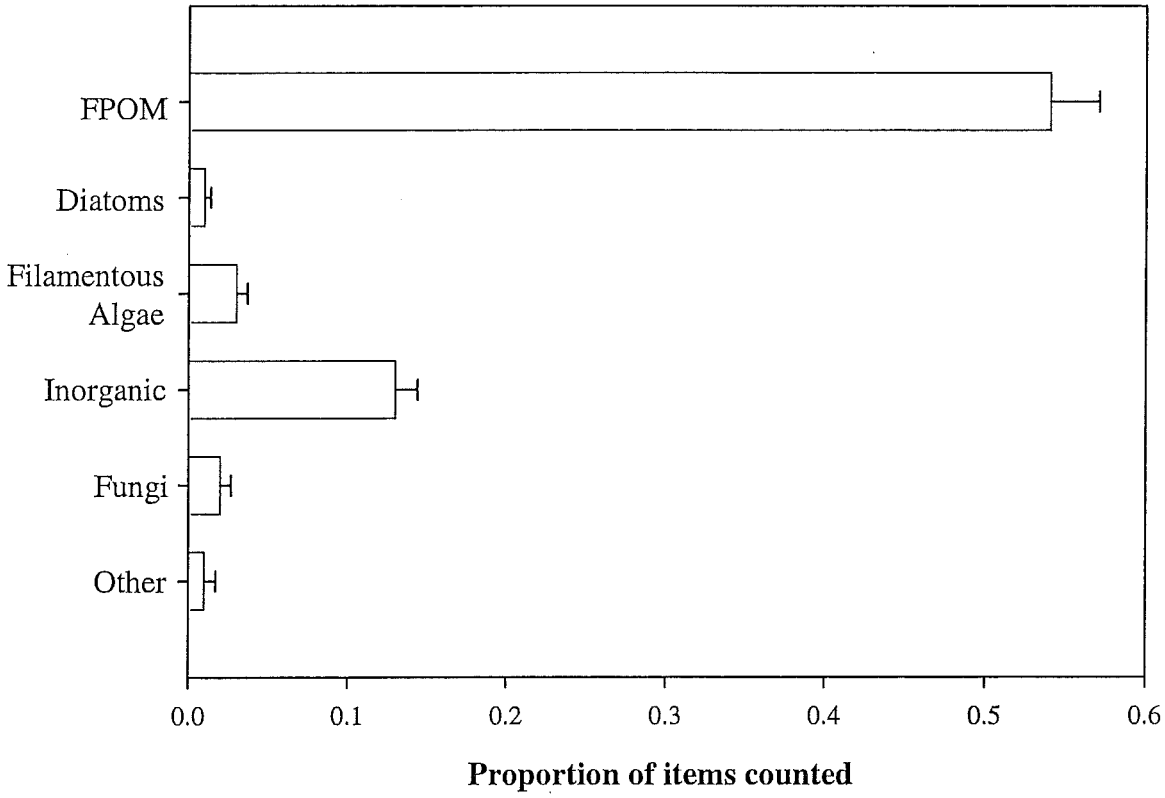


Figure 3.8: Composition of gut contents of ten *Coloburiscus humeralis* nymphs (mean ± 1 S.E.) determined by dissection. The "other" category included animal food items.

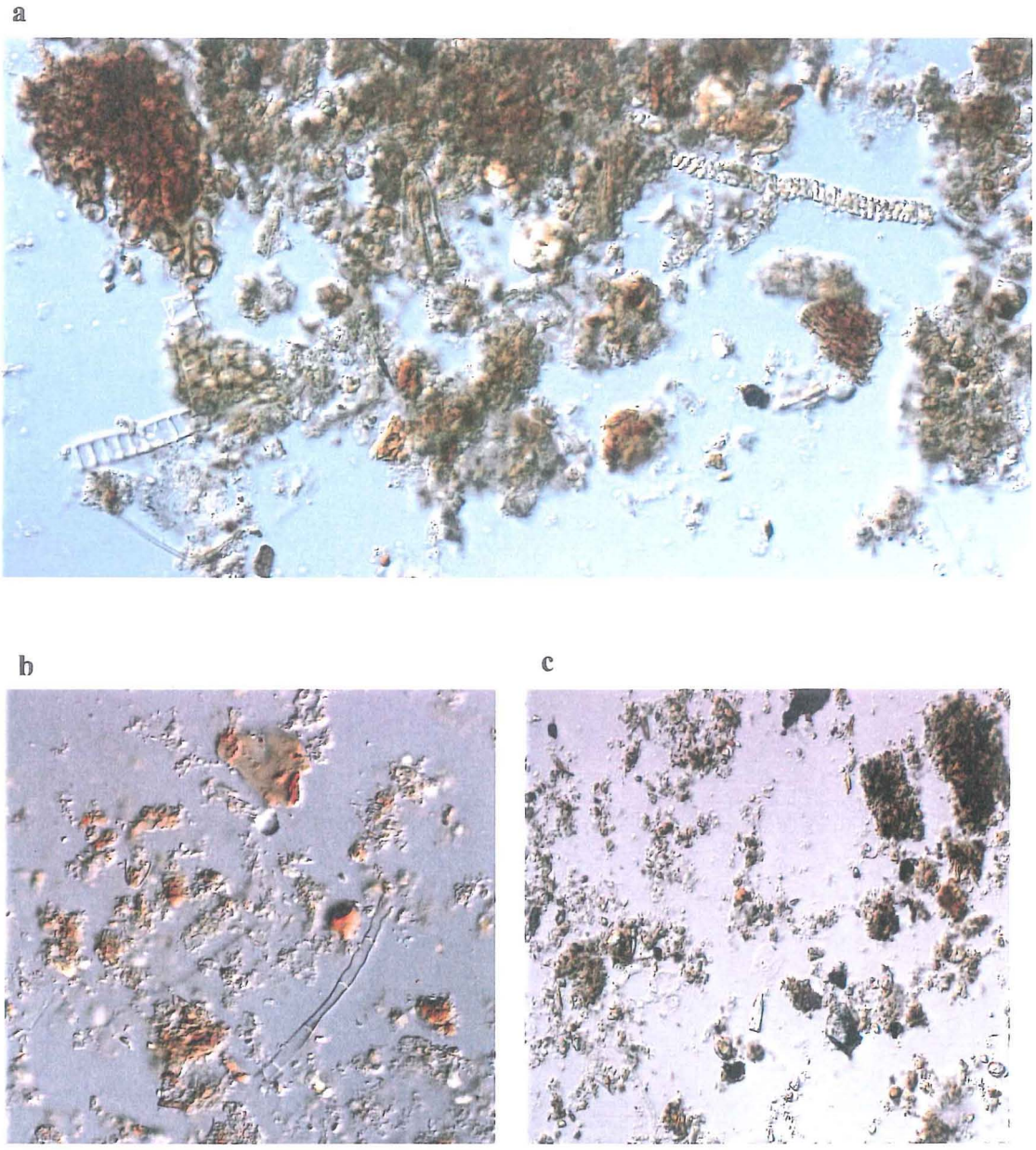


Figure 3.9: Gut contents of three *Coloburiscus humeralis* nymphs. These photographs show amorphous detritus dominating the ingesting material. Note: filamentous algae in (a) and (b).

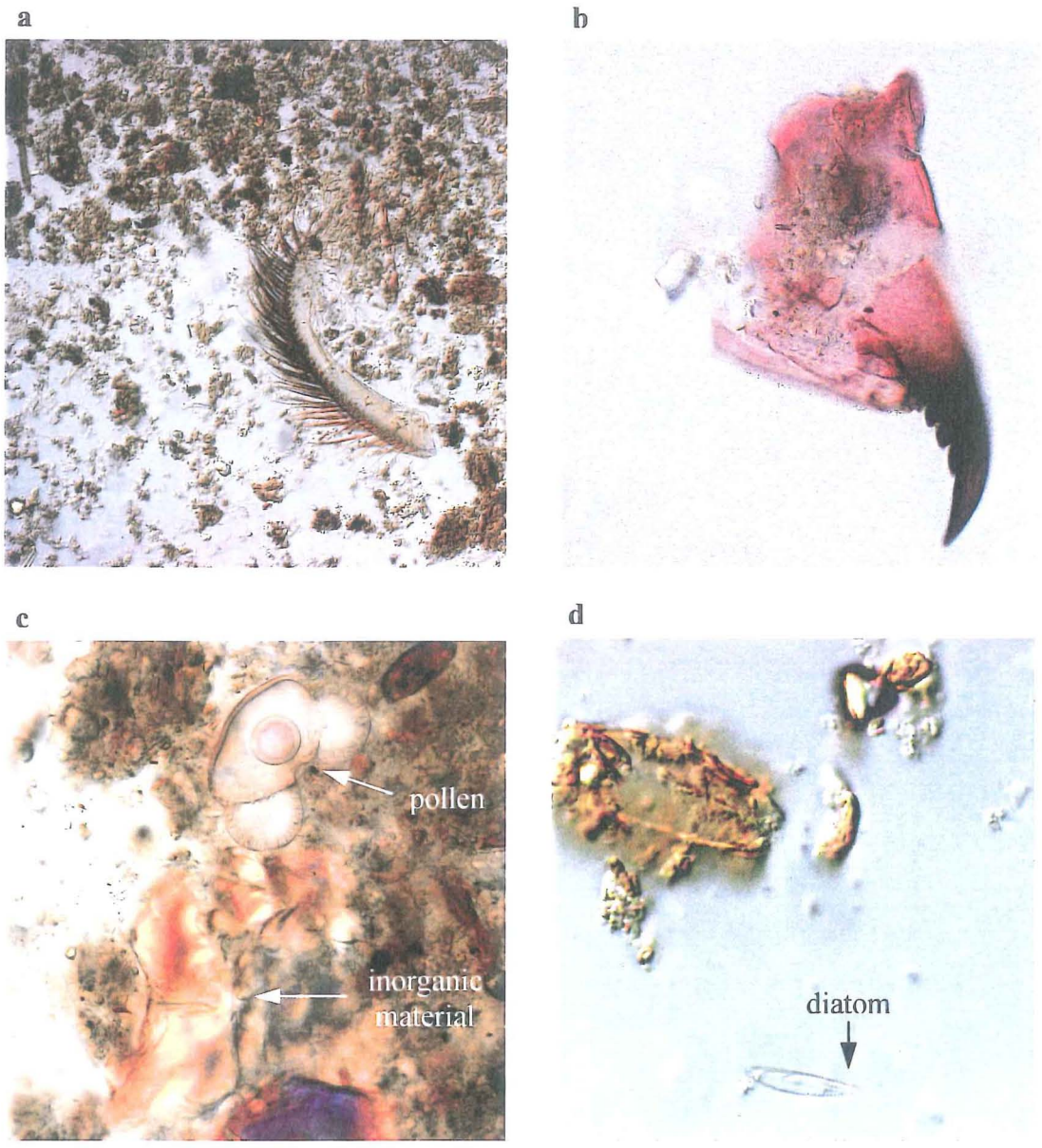


Figure 3.10: Organic detritus dominates gut contents of *Coloburiscus humeralis* but other items also occur in the stomachs of nymphs: (a) and (b) unidentified invertebrate mouthparts, (c) pollen grain and inorganic material, and (d) diatom.

3.3.1 Predatory Fish

The presence of predatory fish had varied effects on the gut fullness of *Coloburiscus* nymphs during the day and at night (Fig. 3.11). During the day, there was no significant effect of predatory trout or galaxiids on gut fullness of *Coloburiscus* nymphs (Table 3.2). The guts of nymphs from all streams were relatively full with approximately 0.15 mg gut DW / mg body DW (Fig. 3.11a).

At night, *Coloburiscus* guts were fullest in streams with no predatory fish (approx. 0.18 mg gut DW / mg body DW) (Fig. 3.11b). In contrast, nymphs from streams with only galaxiids present had the least in their guts, with only 0.10 mg gut DW / mg body DW on average (Fig. 3.11b). However, the influence of galaxiids may have been altered when trout were present. Nymphs from streams with both trout and galaxiids present had fuller guts than nymphs from streams with only galaxiids present (Fig. 3.11, Table 3.2).

Table 3.2: ANOVA testing the effect of predatory galaxiids and trout on the gut contents of *Coloburiscus humeralis* during the day and night.

DAY	**df**	**MS**	**F-ratio**	***p***
Galaxiid	1	0.012	0.095	0.766
Trout	1	0.014	0.110	0.749
Galaxiid x Trout	2	0.098	0.793	0.399
Error	8	0.124		

NIGHT	**df**	**MS**	**F-ratio**	***p***
Galaxiid	1	0.086	0.409	0.540
Trout	1	0.008	0.040	0.847
Galaxiid x Trout	1	1.079	5.128	0.053
Error	8	0.210		

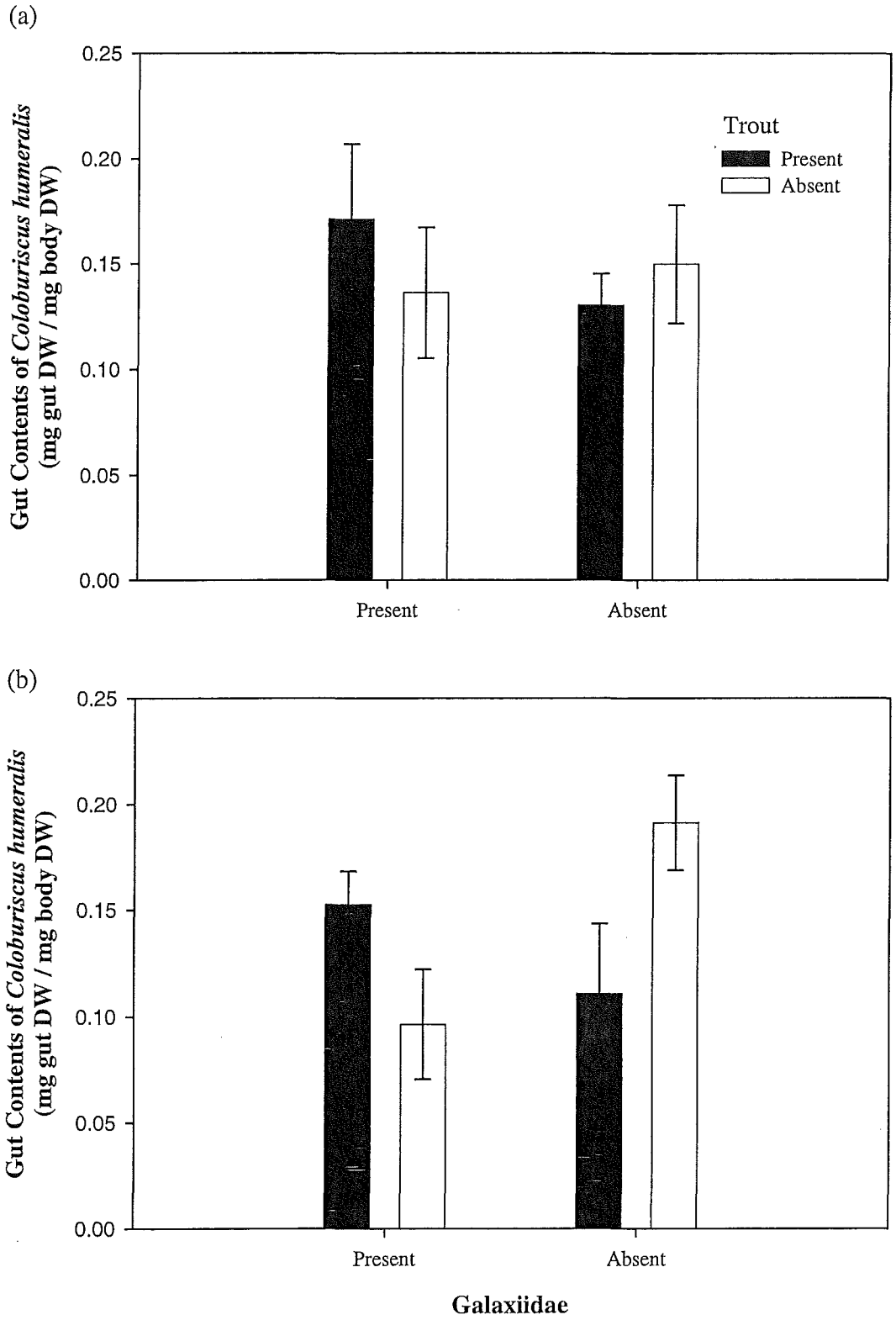


Figure 3.11: Gut fullness of *Coloburiscus humeralis* during the day (a) and night (b) in streams with and without galaxiids, and with and without trout, measured as mg dry weight (DW) gut content per mg DW body weight. Black bars indicate trout present, white bars indicate no trout present. (Error bars = ±1 S.E.).

3.3.2 *Current velocity and food supply*

The inorganic content of seston did not affect gut fullness of *Coloburiscus* nymphs significantly during the day or night ($r = -0.248$, $p > 0.05$) (Fig. 3.12). However, gut fullness of *Coloburiscus* nymphs decreased significantly as organic ($r = -0.752$, $p < 0.05$) and total seston ($r = -0.653$, $p < 0.05$) concentration in the water column increased during the day and night (Fig. 3.13 and 3.14). When gut contents of *Coloburiscus* are examined in relation to the presence of predatory fish and seston concentration, interesting patterns emerge. At night, in streams with predatory galaxiids, there was less in the guts of *Coloburiscus,* even though large quantities of organic seston were present (Fig. 3.13 b). Similar patterns were observed for the total seston data at night (Fig. 3.14 b). During the day, there were no apparent patterns between predatory fish or seston concentration and the gut contents of *Coloburiscus.* Stream current velocity did not have a significant affect on the supply of organic ($r = -0.256$, $p > 0.05$), inorganic ($r = -0.131$, $p > 0.05$) or total seston ($r = -0.174$, $p > 0.05$) in the streams studied (measured as mg DW L^{-1}).

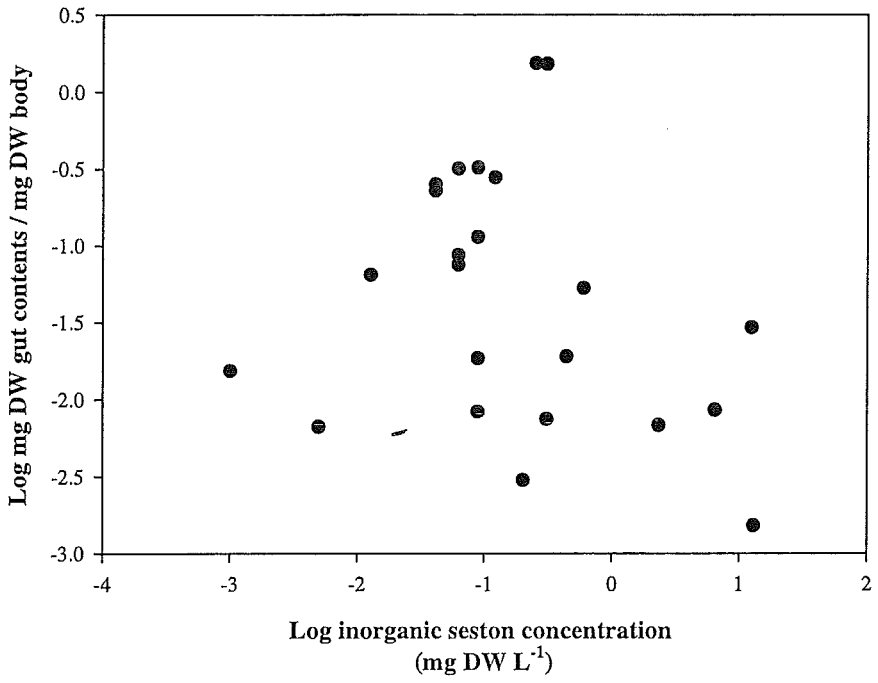


Figure 3.12: Gut fullness (mg DW gut contents / mg DW body) of *Coloburiscus* nymphs in relation to the inorganic seston concentration.

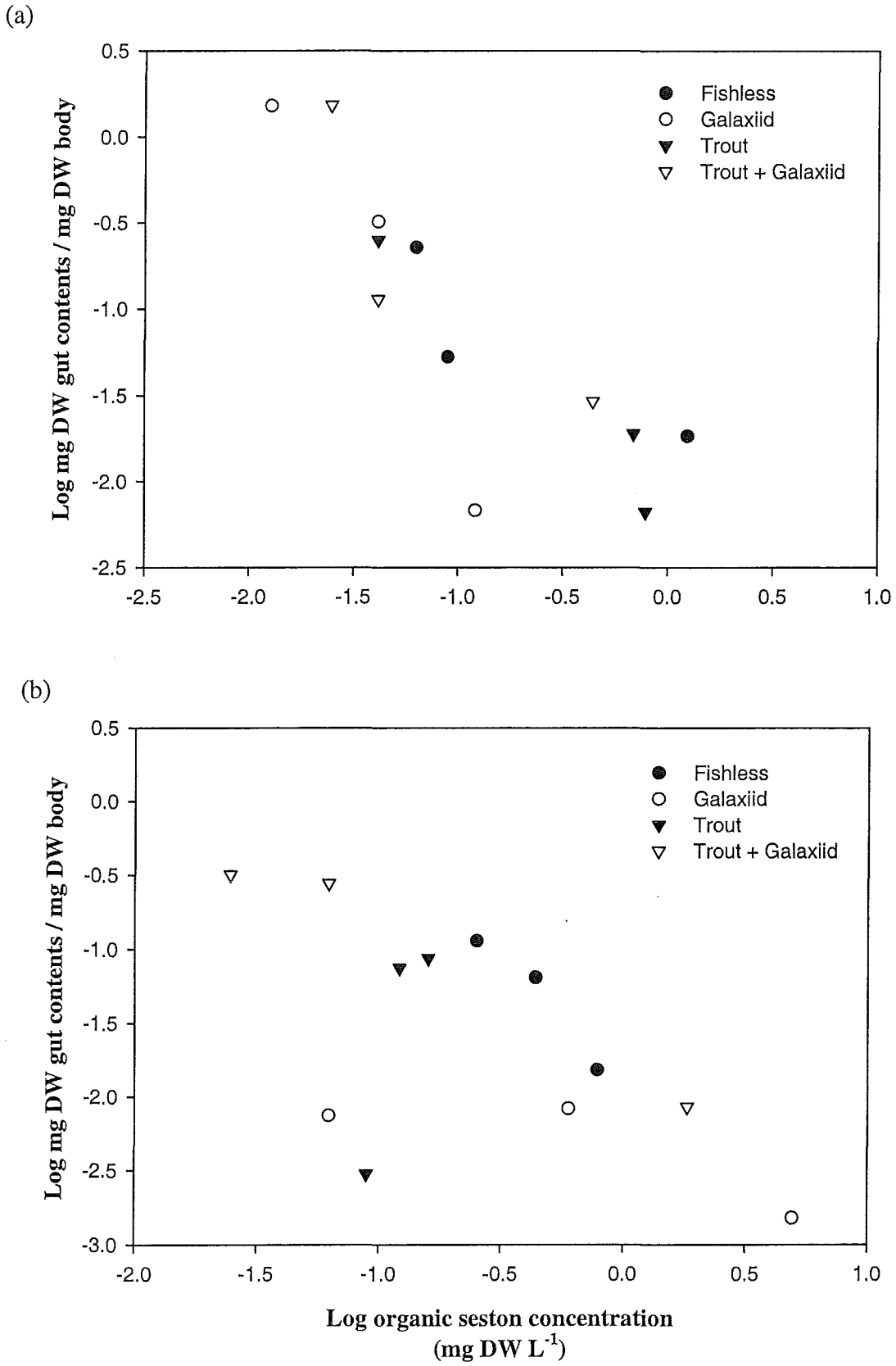


Figure 3.13: Abundance of the organic component of seston in relation to gut fullness (mg DW gut contents / mg DW body) of *Coloburiscus* nymphs during the day (a) and at night (b) in streams with different fish faunas.

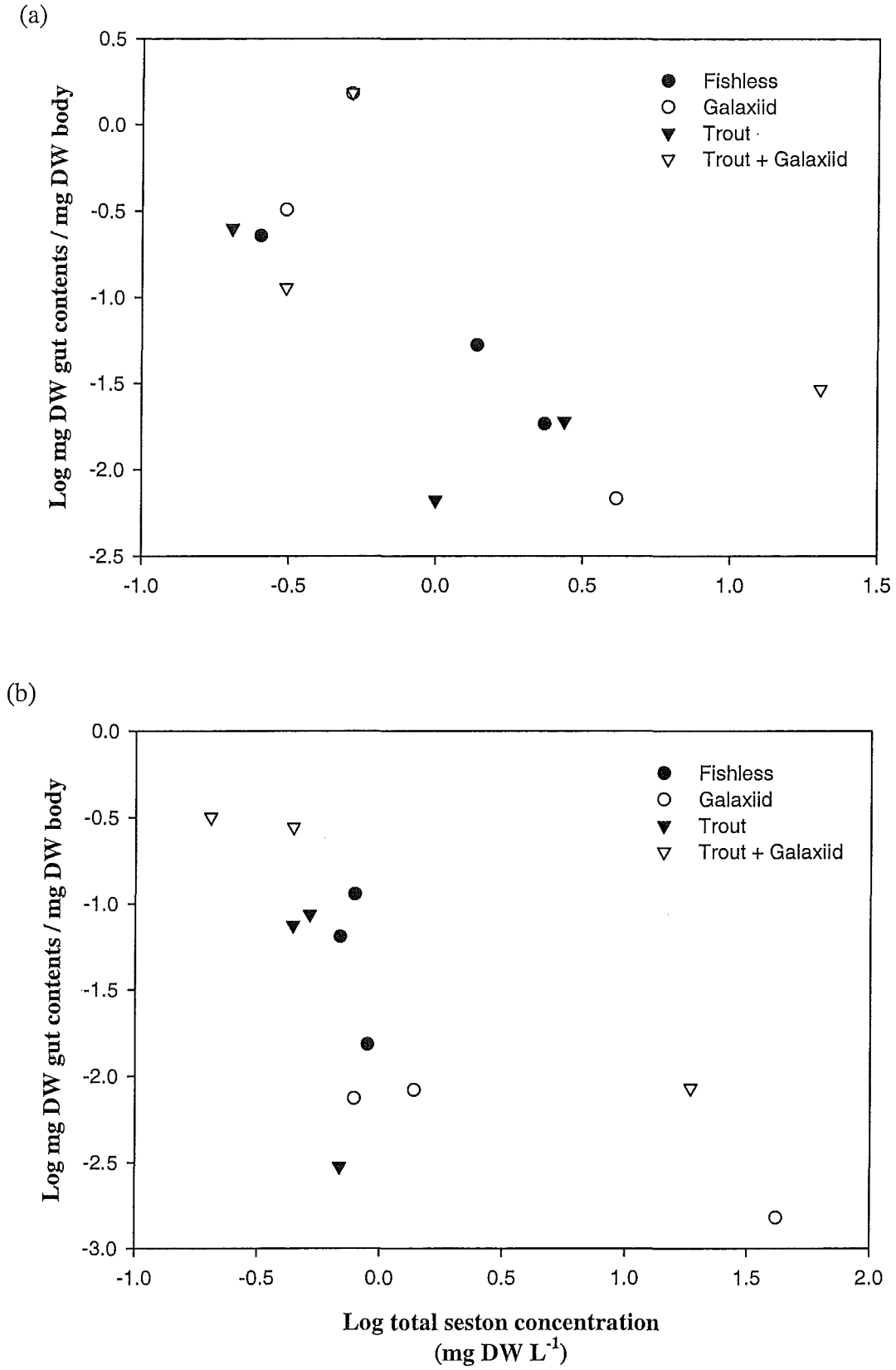


Figure 3.14: Abundance of total seston (inorganic and organic) in relation to gut fullness (mg DW gut contents / mg DW body) of *Coloburiscus* nymphs during the day (a) and at night (b), in streams with different fish faunas.

3.4 DISCUSSION

3.4.1 Food Composition and Supply

The gut contents of *Coloburiscus* nymphs were dominated by organic detritus, consistent with Wisely's (1961) findings. As shown in Chapter Two, nymphs passively collect food particles from the stream current using leg hairs. Therefore, the presence of other food items, including inorganic particles, algae and animal fragments, suggests that nymphs are not discriminatory when feeding. Campbell (1985) also found that the guts of *Coloburiscoides*, a morphologically similar mayfly, were dominated by detritus, but contained a range of food items present.

The food supply of filter feeders is generally influenced by the amount of food available and/or the rate at which it is supplied (Englund, 1991; Finelli, Hart and Merz, 2002). In my study, the organic and total seston concentration in the water column was negatively correlated with gut fullness of *Coloburiscus*. Nymphs had fuller guts at sites where organic and total seston concentrations were low. There are three possible explanations for these unexpected results: (1) food quality is more important than quantity, (2) some factor is preventing feeding in areas of high food availability or (3) results are an artifact of gut content technique whereby empty guts are an indication of higher intake and faster processing of seston.

The quality of an organism's food source may be correlated with feeding rates and gut fullness (Vos et al, 2000), and subsequently growth rates, fecundity and production (Söderström, 1988; Harding, 1997). For example, the presence of filter-feeding hydropsychid and simuliid larvae at lake outlets is related to the availability of a high quality food source at these sites (Wallace and Merritt, 1980; McCreadie and Robertson, 1998). The quality of food available to filter feeders may also depend on the amount of food available, with high quality food at low food levels and low food quality at high food levels (Vos et al., 2000). Therefore, *Coloburiscus* nymphs may have ingested a high quality food source at low concentrations. This explanation seems unlikely, since nymphs would avoid feeding when the abundance of food was high and wait to feed when there was less seston available but it was higher quality.

An alternative hypothesis is that *Coloburiscus* nymphs may be prevented from feeding at times when there are high amounts of seston available. The concentration of seston in the water column, and the supply of seston, influence the feeding

behaviour of filter feeders (Englund, 1991; Voelz and Ward, 1996; Finelli et al., 2002). For example, current velocity and food particle concentration combined to influence food availability in hydropsychid larvae studied by Englund (1991). In other studies, velocity and not food supply have been found to affect ingestion rates of filter feeders, with increased rates of ingestion at higher velocities (Finelli et al., 2002). Therefore, current velocity is relatively important for filter feeders, but at high velocities the ability of some filter-feeding species to feed may be reduced. If higher velocities equate to higher amounts of seston, then it would be beneficial for filter feeders to feed in areas of high velocity. However, morphological adaptations for filter feeding may restrict feeding in certain areas (Malmqvist and Sackmann, 1996). At high levels of seston concentration in this study, current velocity may have exceeded the optimum preferred by *Coloburiscus*. Thus, nymphs may have been prevented from feeding and consequently had less in their guts.

The presence of predatory fish in these streams may also explain why *Coloburiscus* nymphs had less in their guts when the organic component of seston and total seston concentration was high. At night, in streams with native galaxiids, there was a greater concentration of organic and total seston, however nymphs had less in their guts. Therefore, predatory galaxiids may be facilitating the movement of benthic FPOM but also preventing *Coloburiscus* from feeding. Nevertheless, during the day when galaxiids did not appear to affect seston concentration, there was still a negative relationship between seston concentration and gut fullness of *Coloburiscus*. Consequently, other factors may also be affecting the gut contents of *Coloburiscus*.

It is not possible from the results of this study to conclude why seston concentration affected gut fullness of *Coloburiscus* nymphs. However, at high seston concentrations the quality of seston could be lower and nymphs may avoid feeding at these times. The other possibility is that some factor, for example velocity, is preventing nymphs from feeding at high seston concentrations. Predatory fish may also prevent *Coloburiscus* from feeding and this is discussed further in the next section.

3.4.2 Predatory Fish

Predatory fish have negative effects on many invertebrate species, directly via predation or indirectly by altering the behaviour of the prey species (Kerfoot and Sih, 1987). In my study, the effect of predatory galaxiids and trout on the feeding behaviour of *Coloburiscus humeralis* was examined. Introduced trout and native galaxiids had no effect on the feeding behaviour of *Coloburiscus* during the day. Nymphs had relatively full guts, which suggest that predatory fish did not alter the feeding behaviour of *Coloburiscus* during the day. At night, however predatory galaxiids had an impact on the gut fullness of *Coloburiscus* nymphs when they were the only fish in the stream. However, when trout and galaxiids were present together, the effect of galaxiids on the feeding behaviour of *Coloburiscus* nymphs was reduced. Galaxiids may alter the feeding behaviour of *Coloburiscus* by causing nymphs to avoid optimum feeding locations or may generally disrupt their feeding.

Galaxiids are nocturnally active benthic feeders whereas trout feed primarily on items in the drift (Glova and Sagar, 1989a, b; Glova and Sagar, 1991). Dahl and Greenberg (1996) showed that benthic-feeding fish can have a greater impact on the density of benthic invertebrates than drift-feeding fish. They also suggested that benthic-feeding predatory fish may have access to areas beneath stones. If so, *Coloburiscus* nymphs may be at greater risk from predation by galaxiids than trout. Galaxiids may even disturb *Coloburiscus* nymphs from the substrate when swimming near the stream bottom. McIntosh and Townsend (1994) suggested that this was important for *Nesameletus* mayflies.

Some studies of predatory galaxiids have reported low numbers of *Coloburiscus* nymphs in stomach contents (*Galaxias brevipinnis*: Glova and Sagar, 1989, 1991; *G. vulgaris*: Glova and Sagar, 1989). However, these studies were undertaken in rivers where *Coloburiscus* numbers were low. In contrast, Cadwallader (1975) found that *Coloburiscus* constituted approximately 24% and 18% of the diets of *G. vulgaris* and *Gobiomorphus breviceps*, respectively in the Glentui River. The studies of Allibone and Townsend (1998) and Bonnet et al. (1989) six other galaxiid species also indicate that invertebrate benthic abundance corresponds to the percentage in the diet of the fish. However, my predation experiment described in Chapter Four found that no *Coloburiscus* were consumed.

The reduced effect of galaxiids on the feeding behaviour of *Coloburiscus* in streams with trout may be the result of competitive interactions between the two fish species. Trout restrict galaxiids to slower regions of streams at night (McIntosh, Townsend and Crowl, 1992) and had pronounced effects on the activity of native galaxiids (Edge et al., 1993). *Galaxias vulgaris, G. brevipinnis* and *G. paucispondylus* were also absent from areas where large trout were thought to prey on smaller individuals (McIntosh, 2000). Trout may reduce the activity of galaxiids at night and therefore decrease the risk of predation by galaxiids on *Coloburiscus* nymphs.

Predatory fish may also affect the feeding behaviour of *Coloburiscus* indirectly by altering the movements of other invertebrates in the stream. Filter feeders may rely on other invertebrates to supply FPOM, either directly by breaking CPOM down into FPOM, or via their movements in the stream. Wallace et al. (1991) found that macroinvertebrates facilitated the movement of FPOM in three North Carolina streams. Therefore, if the movement of stream invertebrates was important as a source of FPOM for *Coloburiscus* and predatory fish were altering this supply, then we would expect to see a reduction in the amount of seston in streams with predatory fish. I found that the supply of seston was higher at night in streams with predatory galaxiids. This suggests that galaxiids rather than invertebrates have a greater role in facilitating the transport of FPOM available to *Coloburiscus*.

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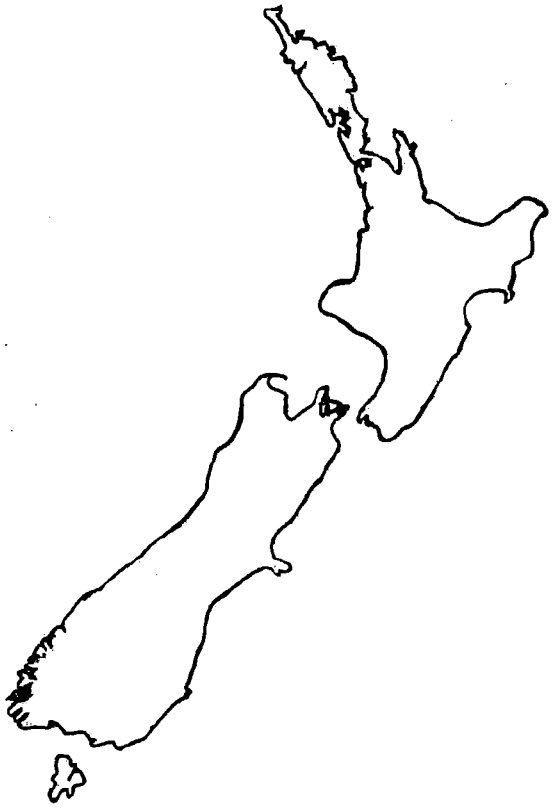

CHAPTER

CHAPTER FOUR

Distribution and Abundance of *Coloburiscus humeralis*

4.1 INTRODUCTION

Many factors influence the distribution of benthic stream invertebrates including a combination of abiotic and biotic factors (see Hynes, 1970). Biological variables, such as predation and competition, may be altered by environmental factors (Peckarsky, 1983). For example, abiotic factors may be more important determinants of invertebrate community structure in streams that experience high levels of disturbance (Fig. 4.1) (Peckarsky, 1983). Conversely, community structure in benign environments may be controlled by biotic interactions (Fig. 4.1). The factors controlling different species will depend on feeding mode, position in the food chain and habitat requirements (e.g. Ciborowski, 1983; Englund, 1991; Voelz and Ward, 1996). The purpose of this chapter is to examine the distribution of the filter-feeding mayfly, *Coloburiscus humeralis*, in relation to abiotic and biotic variables.

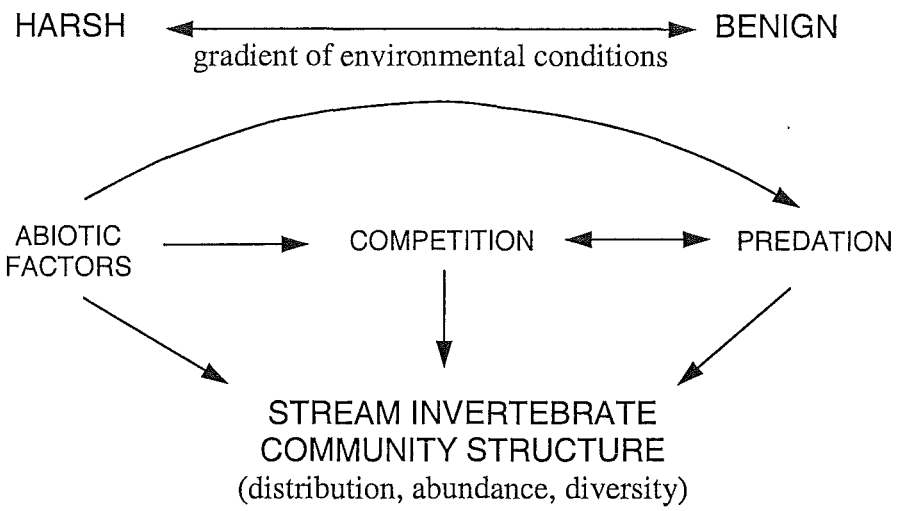

Figure 4.1: Hypothetical model proposed by Peckarsky (1993) to explain invertebrate community structure.

4.1.1 Abiotic Variables

Physical variables have been implicated in the community structure of many New Zealand stream invertebrates (Winterbourn, Rounick and Cowie, 1981). However, the predominance of studies in this area of stream ecology and the lack of studies examining biotic interactions may explain this (Thomson and Townsend, 2001). Below I summarise the results of some of the studies that have examined abiotic factors in relation to the distribution of stream invertebrates, with particular emphasise on filter-feeding species.

Stream current is one of the major defining factors of stream ecosystems (Hynes, 1970) and many studies have examined the effect of current velocity on the distribution of stream invertebrates (e.g. Jowett and Richardson, 1990; Collier, 1994; Malmqvist and Sackmann, 1996). Stream invertebrates may rely on stream current for feeding purposes or respiratory requirements (Hynes, 1970). For example, larger individuals of the grazing mayfly *Deleatidium* have been shown to occupy faster water because of increased amounts of oxygen, as well as increased nutrient exchange with algae at higher velocities (Jowett and Richardson, 1990; Collier, 1994). Current velocity also controls the food supply of filter-feeding species and may influence interactions with other invertebrate species. Malmqvist and Sackmann (1996) found that some predatory invertebrate species were less efficient at higher current

velocities, and that this allowed their prey, larval blackflies, to avoid predation by selecting habitats at higher velocity. *Coloburiscus humeralis* is a passive filter-feeder which is dependent on stream current to supply FPOM. Observations of *Coloburiscus* from previous studies suggest that nymphs inhabit fast flowing sections of streams (Wisely, 1962). Therefore, changes in velocity may alter the food supply of *Coloburiscus* in streams and preferences for faster velocities may exist.

Substrate is another important component of stream ecosystems, which provides habitat and refuge for stream invertebrates (see Minshall, 1984). Many invertebrate species prefer larger substrates because they provide a stable habitat (Quinn and Hickey, 1990). However, species like *Ichthybotus* may show preferences for smaller substrates (sand and silt) because they live in burrows in soft sediments (Winterbourn, Gregson and Dolphin, 2000). Many filter-feeding species use substrate to attach themselves (simuliid larvae) or their nets (hydropsychid larvae) to collect FPOM. Therefore, preferences for large, stable substrates are not surprising.

Coloburiscus uses long hairs on the femur and tibia of the legs to collect food particles from the current. Therefore, stable habitat may be important for *Coloburiscus*, since nymphs may not be able to feed so efficiently in unstable environments. *Coloburiscus* nymphs may show preferences for larger substrates in streams. Substrate may also provide refuge from predation. Larger substrates may provide more refuge from predatory fish than smaller substrates. Consequently, *Coloburiscus* may also select larger substrates to reduce the risk of predation by predatory fish.

Many other abiotic factors are likely to affect the distribution of *Coloburiscus humeralis* in streams, as shown by past studies (e.g. Wisely, 1962; Harding and Winterbourn, 1993). In this chapter, a field survey was used to examine a variety of abiotic factors in relation to the distribution of *Coloburiscus* in streams at Cass, Hanmer and on Banks Peninsula. An in-stream channel experiment was also used to examine the interactive effects of disturbance and predation on the abundance of *Coloburiscus* nymphs.

4.1.2 Biotic Variables

Biotic factors, such as predation and food supply influence the distribution of many stream invertebrates (see McIntosh, 2000). In many cases these studies have examined grazing invertebrates and fish predators in algal-based systems (Peckarsky et al., 1993; McIntosh and Townsend, 1994, 1995, 1996). In some detrital-based systems, predatory fish affect the community structure of benthic invertebrates (Flecker, 1984), but in others little or no effect has been found (Reice, 1991; Rosenfeld, 2000). Therefore, it is important when examining the effect of predatory fish on a prey species to consider the effect of other factors, such as the prey species' position in the food chain or feeding mode.

Predation

Predation is another important factor influencing the population dynamics and community structure of stream ecosystems (see Zaret, 1980; Kerfoot and Sih, 1987). Predatory species may control prey populations directly via consumption, or indirectly by altering a prey species' behaviour, morphology or life history (Sih, 1987). Additionally, the effect of predatory fish on the distribution of a prey species may be different to that of predatory invertebrates.

The impact of predatory fish on a prey species will depend on the foraging behaviour of the fish. In New Zealand streams, two primary foraging strategies exist, drift-feeding and benthic-feeding. Introduced trout consume a wide range of prey species but generally drifting invertebrate species dominate the diet (Allan, 1978; Sagar and Glova, 1995). In contrast, native galaxiids consume benthic and drifting invertebrates (Cadwallader, 1975; Glova and Sagar, 1991; Glova and Sagar, 1993). Therefore, the risk of predation for benthic invertebrates may be higher in streams with predatory galaxiids. *Coloburiscus humeralis* nymphs are often associated with the undersides of rocks (Wisely, 1962) where there may be a low risk of predation by fish. However, native galaxiids may be able to exploit *Coloburiscus* populations because of their benthic feeding strategy.

Predatory invertebrates may present a greater risk to benthic invertebrate species than fish because they are able to move into areas where invertebrates may find refuge from predatory fish. The size of invertebrate prey species may, however,

prevent predatory invertebrates from consuming larger species. For example, *Coloburiscus* is a relatively large mayfly, therefore many smaller predatory invertebrates would present no risk to *Coloburiscus* nymphs. However, large predatory invertebrates, such as *Archichauliodes*, may be able to exploit *Coloburiscus* populations.

Many prey species have also evolved behavioural and morphological defences to combat predators. Predatory species may not appear to affect prey populations via changes in abundance. However, indirect effects, such as changes in behaviour or morphology of the prey species, may occur. This is examined further in Chapter Five.

Food Supply

"Resource acquisition is a constant requirement of all organisms and, in a sense, provides an absolute limit to population density" (p 3: O'Brien, 1987). Many factors will influence the supply of food to invertebrate species, including both abiotic and biotic variables. Grazing invertebrate species feed on algae, which requires certain levels of light and oxygen to grow. Similarly, predatory fish and invertebrates may restrict grazing invertebrates from feeding. Therefore, it is important to examine not only the amount of food available to a species but also factors which may alter food availability.

Filter-feeding invertebrates feed on a relatively abundant but poor quality food source (FPOM), whose supply is determined by current velocity. Therefore, associations with velocity are common in filter feeders (Englund, 1991; Malmqvist and Sackmann, 1996). Many filter-feeding species are also associated with lake outlets, where there may be a source of high quality seston (Wallace and Merritt, 1980; McCreadie and Robertson, 1998). The distribution of *Coloburiscus* in streams may not be greatly altered by changes in food supply because FPOM is relatively abundant in most streams. However, the quality of seston in streams may alter the growth rates of *Coloburiscus*, although this was not examined in this study.

In this study, I examined factors influencing the distribution and abundance of *Coloburiscus humeralis* in streams. Streams in the Cass - Craigieburn region of the Southern Alps, Banks Peninsula and Hanmer Springs, Canterbury were surveyed and

classified based on the presence of predatory fish to examine their effect on *Coloburiscus* distribution and abundance. I also investigated (1) whether abiotic variables influenced the distribution of *Coloburiscus,* (2) associations between *Coloburiscus* and other invertebrate species and (3) the combined effects of predatory fish and disturbance on the abundance of *Coloburiscus*, using an in-stream channel experiment.

4.2 METHODS

4.2.1 Survey Methods

Invertebrates samples were taken from sites at Cass, Hanmer and Banks Peninsula during winter 2001 (Cass: 1 September; Banks Peninsula: 19 July; Hanmer: 12 September). Sample sites are described further in Chapter Three. Five samples were taken at random points within a 50 m reach of each stream with a Surber sampler (0.0625m^2, 250 μm mesh) and preserved individually with 75% ethanol. All invertebrate species were sorted from each sample and counted. To examine patterns of distribution of *Coloburiscus* nymphs in relation to size, their body length was measured as described in Chapter Three.

Velocity, depth and substrate composition were determined at each Surber sample site. Depth was measured with a metre ruler at the centre of the Surber frame. Velocity was measured at two thirds depth of the stream for 20 seconds (EDSC NIWAR Current Meter Counter).

Surface substratum composition was assessed visually in a one metre area around the site where each Surber sample was taken. Substrata were classified as: boulders (25+ cm), large cobbles (13 – 24 cm), small cobbles (6 – 12cm), gravel (2 – 5 cm) and sand (<2cm); proportional composition of each fraction was determined. The proportional data were used to calculate the index of Quinn and Hickey (1990) for each site. This involves summing the mid-point value for each substratum fraction multiplied by their proportional values.

Dissolved oxygen and water temperature were measured with a YSI 95 Dissolved Oxygen Meter between 10 am and 3 pm by placing the probe randomly in a riffle section of the stream and holding it there until the readings stabilized.

A two litre water sample was taken to determine nitrate-N, phosphate-P, alkalinity, pH and conductivity in a pre-washed plastic container from each stream by fully immersing the container in a relatively fast flowing section of the stream. Water samples were taken back to the laboratory and refrigerated for up to 3 days before analyses were made. Nitrate-N and phosphate-P were determined using the Hach (1992) DR/2000 direct reading spectrophotometer procedure. Alkalinity was measured by titration with 0.01 N HCl (Mackereth, 1963). A 100 ml water sample (plus indicator) was titrated with a measured volume of HCl until the solution turned clear. The volume of HCl used was multiplied by 5 to obtain alkalinity as mgL^{-1} $CaCO_3$. Conductivity (HANNA instruments HI 8333 conductivity meter) and pH (calibrated Solstat FET pH meter EPM-100) were measured with probes in a 30 ml sample of water. Stream substratum stability was assessed at each stream using the bottom component of the Pfankuch Index (Collier, 1992). The bottom section of the Pfankuch Index measures stream channel stability by assessing factors that include substrate size, shape and packing (Pfankuch, 1975). Percentage of overhead riparian vegetation was assessed visually at each stream. Results are summarised in Table 4.1.

4.2.2 Statistical Methods

A two-way factorial ANOVA with blocks was used to determine the effect of predatory fish on abundance of *Coloburiscus*. The treatments were trout (presence or absence) and galaxiid (presence or absence). Region was treated as a block to examine differences between the three regions. Data were $\log_e$ transformed to conform to the assumptions of ANOVA.

Discriminant function analysis was used as an exploratory data technique to identify abiotic and biotic factors influencing the presence or absence of *Coloburiscus* in streams. The data were transformed (Table 4.2) and *Coloburiscus* presence/absence was used as the grouping variable. Factors identified as important were then tested using Pearson correlation to identify potential relationships with *Coloburiscus* density.

Table 4.2: Data transformations for abiotic and biotic variables used for statistical analysis. – indicates no transformation.

Variable	Transformation
Coloburiscus density	ln (x + 1)
Altitude	-
Slope	ln (x + 1)
Fish Biomass	$\log_{10}$
Mean No.	ln (x + 1)
Stability	$\log_{10}$
Substrate Index	-
FPOM (< 75mm)	ln
Velocity	-
Alkalinity	-
Nitrate-N	ln
Phosphate-P	ln
pH	-
Conductivity	-
Dissolved Oxygen	-
Depth	ln
Temp	-
Channel Width	ln

I used PCA to establish which invertebrate species were related to *Coloburiscus* abundance. Principal Components Analysis (PCA) is a method of decomposing a correlation matrix (SYSTAT, 2001). It allows large groups of variables to be studied by grouping into "factors" and examining correlations between factors (SYSTAT, 2001). PCA was preformed on invertebrate abundance data and Pearson correlation was used subsequently to test for relationships between the "factors" and *Coloburiscus* abundance.

4.2.3 Channel Experiment

During March 2001 (1 – 15 March) 24 in-stream channels were used to examine the interactive effects of predatory fish and disturbance on *Coloburiscus humeralis*. The channels were located in a 200 m reach of Grasmere Stream and were made from 1.5 m sections of PVC pipe (25 cm) cut longitudinally in half. Six millimetre mesh was secured at end of each channel to allow emigration and immigration of stream invertebrates but prevent the movement of predatory fish. Mesh (3 mm) on the tops of the channels also prevented fish from jumping out. A layer of gravel and 16 rocks from a dry river bed were added to each channel. Each channel was placed approximately 20 cm above the stream bed, with its top just out of the water. The channels were set up on 22 – 23 February 2001 and left for a week so that algae could colonize. A randomized block design was used, with a block consisting of two groups of four channels (Fig. 4.2). Predator and disturbance treatments were designated randomly.

After the one week algal colonization period invertebrates were added to the channels. Five Surber samples ($0.0625m^2$, 250 μm mesh) collected from riffles below the experiment area were emptied into each channel. The next morning the substrata in twelve channels was disturbed using hand tumbling for a two minute period, to simulate a natural disturbance. Immediately following this, a small Surber sample ($0.0225m^2$, 250 μm) was taken from every channel. On the same day (approximately 4.30 pm) predatory fish were introduced.

Native galaxiids (*Galaxias vulgaris*) and brown trout (*Salmo trutta*) were collected from two streams in the Cass region. Fish were of similar lengths (mean fork length: trout, 8.9 ± 0.7 mm; galaxiid, 7.9 ± 0.9 mm) but trout were heavier (trout: 7.3 ± 1.4 g; galaxiids 3.9 ± 1.4 g).

The experiment ended on 15 March, when the fish were caught and killed with an overdose of anaesthetic (2-phenoxyethanol). Fish were weighed, measured and frozen for later gut examination. All invertebrates in the channels were washed into a net (250 μm mesh) and preserved in ethanol. Invertebrates were identified using Winterbourn, Gregson and Dolphin (2000). All *Coloburiscus* nymphs found were dried for 24 hr at 65 °C and weighed to the nearest 10 μg on a Cahn microbalance. *Coloburiscus* samples were then ashed in a muffle furnace at 450

°C for one hour and reweighed to determine ash free dry mass for each channel. Data were log transformed and a two- way ANOVA with block was used to test the effects of predation and disturbance on the abundance and AFDM of *Coloburiscus humeralis*.

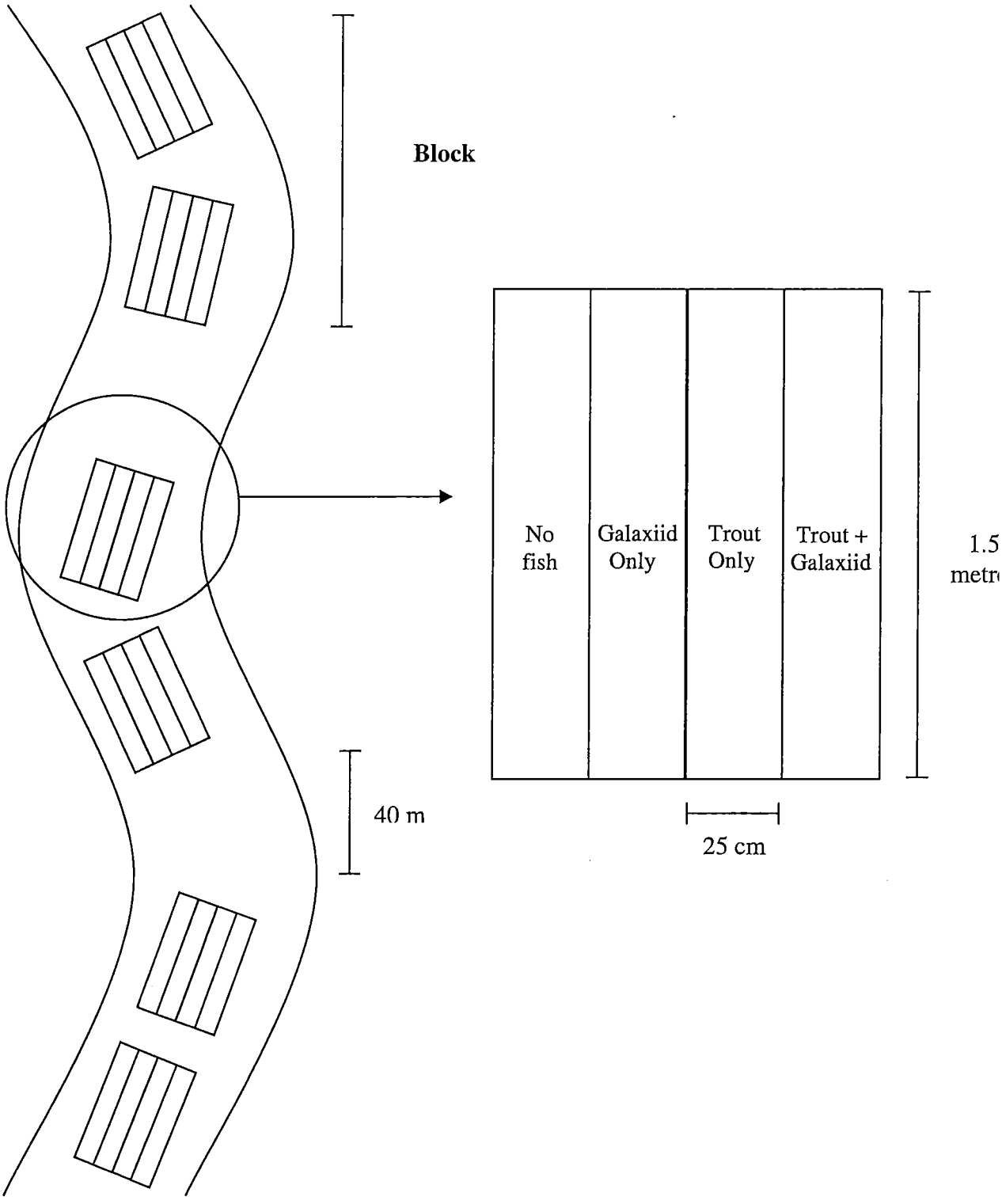

Figure 4.2: Setup and design of stream channels to examine the effect of predatory fish and disturbance on *Coloburiscus humeralis*. Note: not to scale.

4.3 RESULTS

4.3.1 Distribution Survey

Coloburiscus nymphs were most abundant in streams with introduced trout and least common in fishless streams (Fig. 4.3). However, there was no significant difference in the abundance of *Coloburiscus* nymphs between any of the predatory fish regimes (Table 4.3). Likewise, abundance of nymphs was not significantly different between regions (Table 4.3).

Table 4.3: ANOVA examining the effect of predatory fish (either trout or native galaxiids presence or absence) and region (Cass, Hanmer and Banks Peninsula) on the ln (x +1) transformed abundance of *Coloburiscus humeralis*.

	df	MS	F-ratio	*p*
Region	2	12.417	2.509	0.113
Trout	1	14.541	2.938	0.106
Galaxiid	1	1.209	0.244	0.628
Trout x Galaxiid	1	5.461	1.103	0.309
Error	16	4.949		

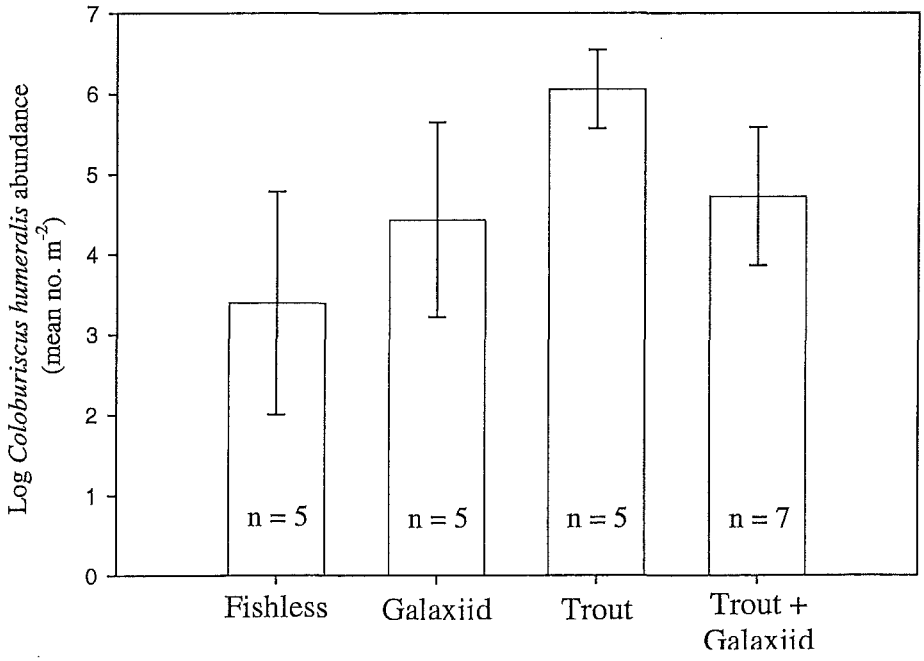

Figure 4.3: Abundance of *Coloburiscus humeralis* in streams with galaxiids and trout present or absent (mean + S.E.).

Discriminant function analysis was used to determine which habitat variables (Table 4.4) were most useful for discriminating between *Coloburiscus* presence or absence. Bed stability was the only factor significantly associated with the presence of *Coloburiscus* in streams from the three geographic regions. The abundance of *Coloburiscus* was positively related to stream channel stability as measured by the Pfankuch score ($r = -0.742$, $p < 0.05$, note lower score indicates higher stability) and mean abundance of *Coloburiscus* nymphs was generally higher in the more stable streams (Fig. 4.4).

Table 4.4: Discriminant functions analysis testing the effect of habitat variables on the presence of *Coloburiscus humeralis*.

% Correctly Classified	Classification	Abs.	100%
		Pres.	75%
	Jackknifed Classification	Abs.	50%
		Pres.	75%

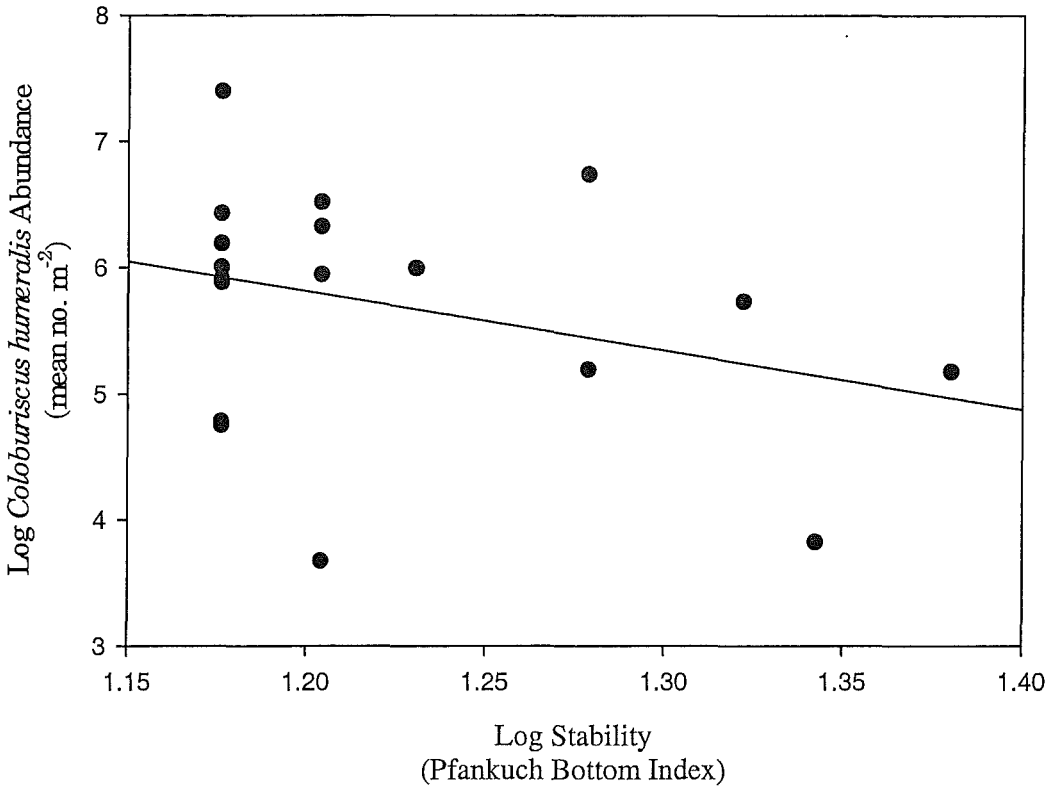

Figure 4.4: Abundance of *Coloburiscus humeralis* in streams at Cass, Hanmer and Banks Peninsula in relation to stream bed stability. Note: The larger the stability index value, the more unstable the stream.

There was no significant relationship between the size of *Coloburiscus* nymphs and substrate size ($r = 0.438$, $p > 0.05$), however larger nymphs tended to be found on larger substrates (Fig. 4.5). Nymph size was negatively associated with velocity ($r = -0.50$, $p < 0.05$) (Fig. 4.6) and depth ($r = -0.49$, $p < 0.05$) (Fig. 4.7), respectively. Thus large nymphs were generally found at slower current speeds and in shallower water than smaller nymphs.

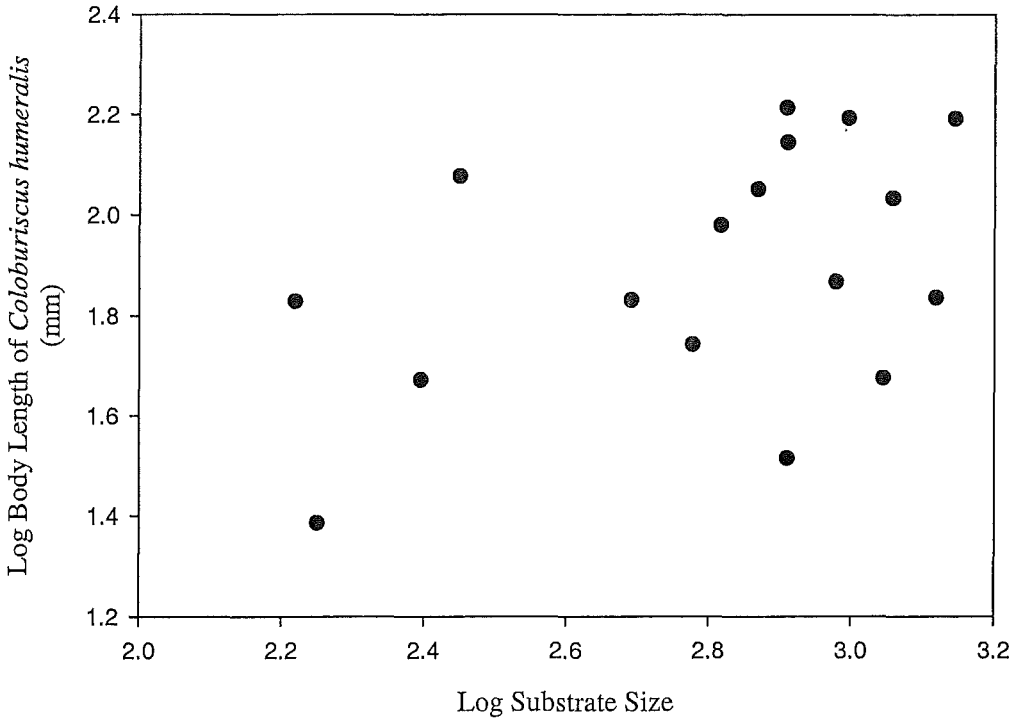

Figure 4.5: Size of *Coloburiscus* nymphs (mm body length) in relation to substrate size index ($r = 0.438$, $p > 0.05$).

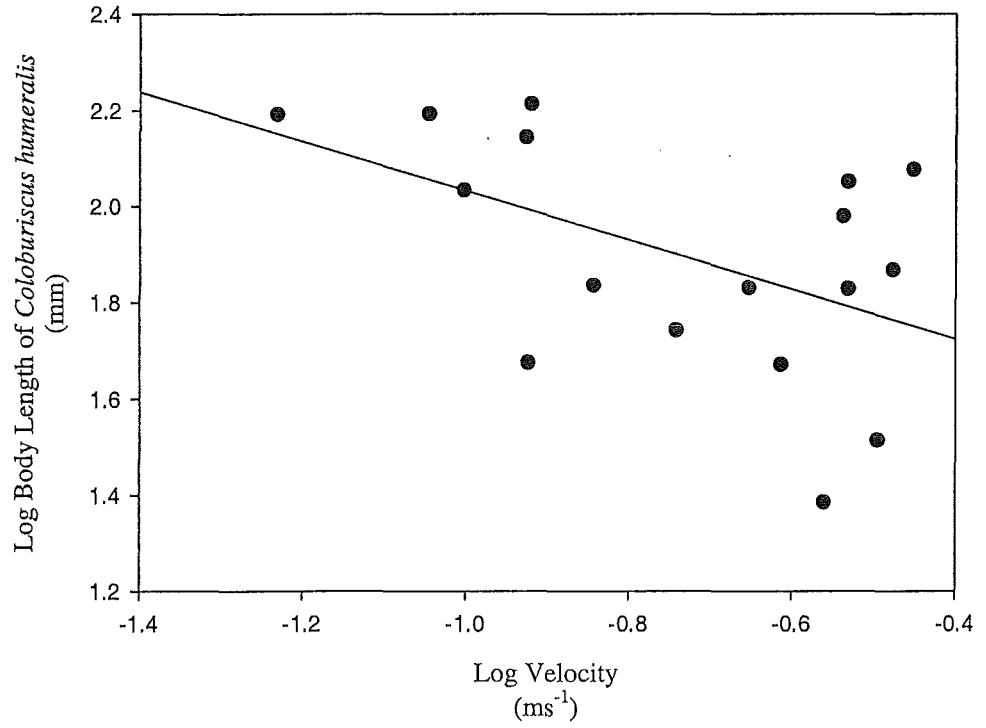

Figure 4.6: Size of *Coloburiscus* nymphs (mm body length) in relation to velocity ($r = -0.50$, $p < 0.05$).

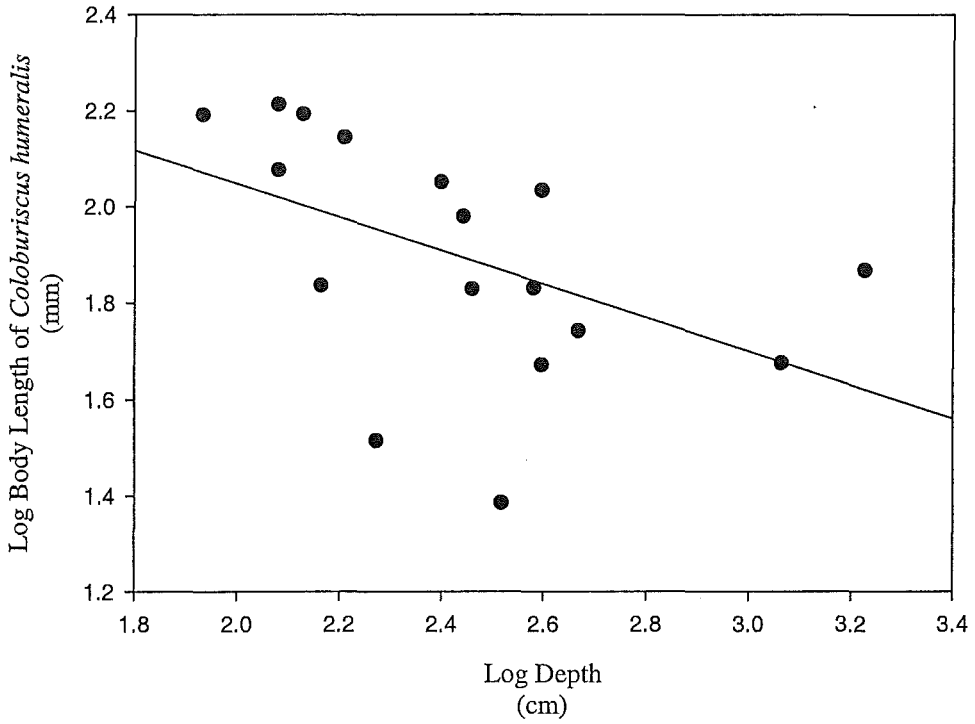

Figure 4.7: Size of *Coloburiscus* nymphs (mm body length) in relation to stream depth ($r = -0.49$, $p < 0.05$).

PCA reduced the list of 58 invertebrate taxa to four factors. Factors one and two were significantly correlated with *Coloburiscus* abundance and explained 17 and 14 % of total variance, respectively. Fifteen invertebrate taxa were associated with factor one which was negatively correlated with *Coloburiscus* abundance ($r = -0.688$, $p < 0.05$) (Fig. 4.8). Factor two, which incorporated nine taxa, was positively correlated with *Coloburiscus* abundance ($r = 0.463$, $p < 0.05$) (Fig. 4.9).

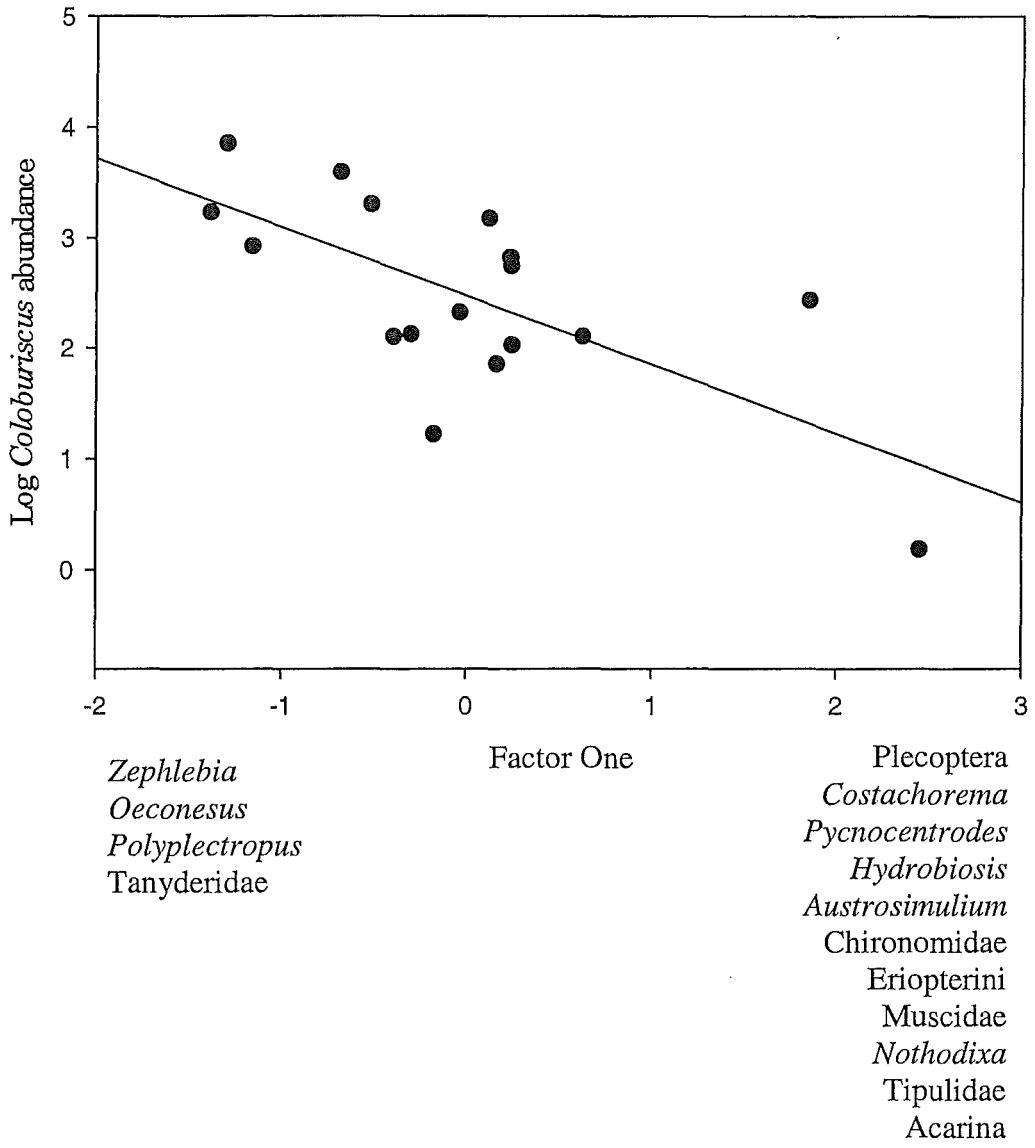

Figure 4.8: *Coloburiscus* abundance in relation to PCA factor one. Invertebrate species at the negative and positive ends of the x-axis have negative and positive correlations with the axis, respectively.

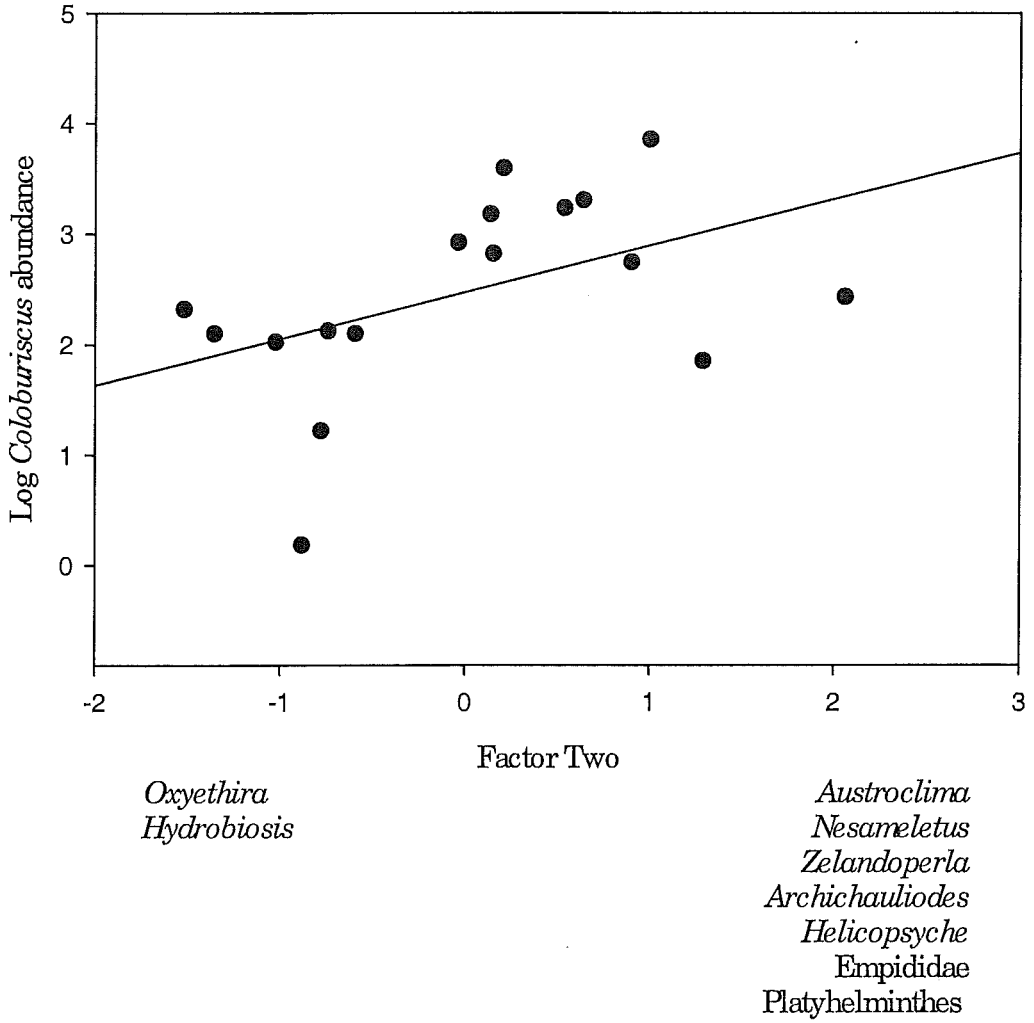

Figure 4.9: *Coloburiscus* abundance in relation to PCA factor two. Invertebrate species at the positive and negative ends of the axis are positively or negatively correlated with the axis.

4.3.2 Channel Experiment

Coloburiscus nymphs were relatively abundant in the channels, and made up 8.1 ± 1.2% of total invertebrate numbers. However, predatory fish in the channels had no effect on the biomass of *Coloburiscus* (Table 4.5). No *Coloburiscus* were found in the gut contents of the predatory fish, despite most fish having relatively full guts (mean # prey items/fish = 6). However, disturbance significantly affected the biomass (AFDM) of *Coloburiscus* (Table 4.5). The AFDM (Fig. 4.10) of *Coloburiscus* was lower in the disturbed channels and there was no interaction involving fish.

Table 4.5: ANOVA examining the effect of predatory fish and disturbance on the biomass of *Coloburiscus humeralis* in a stream channel experiment.

	df	**MS**	**F**	***p***
Block	2	3.039	4.65	0.02
Disturbance	1	3.039	12.76	0.003
Predator	3	0.483	0.74	0.55
Dist. * Pred.	3	0.209	0.32	0.81
Error	14	0.653		

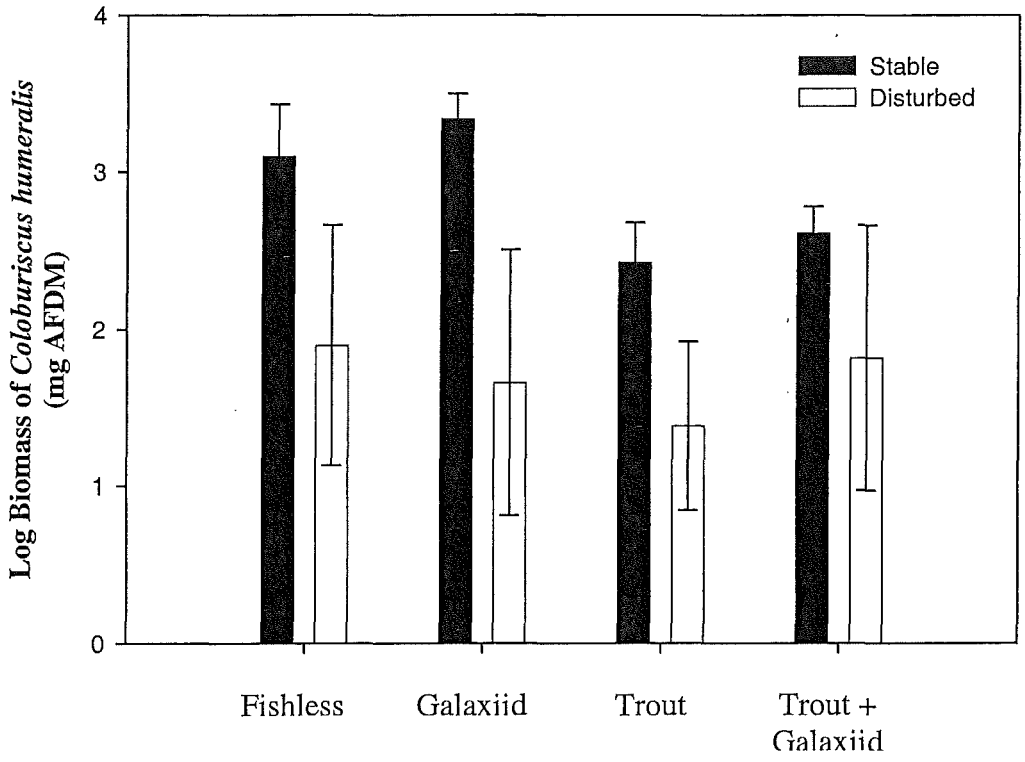

Figure 4.10: Biomass of *Coloburiscus humeralis* (mg AFDM) in channels with and without trout and galaxiids and with two disturbance regimes.

4.4 DISCUSSION

4.4.1 Predation

Many factors influence the abundance and distribution of freshwater macro-invertebrates (see Hynes, 1970). However, factors affecting the distribution and abundance of filter-feeding invertebrates are not well understood. In many ecosystems, predators are important determinants of prey abundance, distribution and feeding behaviour (see Zaret, 1980; Kerfoot and Sih, 1987). However, results of may field survey and channel experiment indicated that introduced trout and native galaxiids had no significant effect on the abundance of *Coloburiscus*. Slightly higher numbers of *Coloburiscus* were found in streams with trout, but this may simply mean that trout and *Coloburiscus* both prefer stable streams. The channel experiment also

supported these results, with no effect of predatory fish on the biomass of *Coloburiscus*. The absence of *Coloburiscus* from the guts of trout and galaxiids in the channel experiment suggests that these fish species do not consume nymphs.

Predatory fish do not appear to consume *Coloburiscus* and therefore they may not affect the abundance of nymphs. In streams, *Coloburiscus* nymphs are found on the undersides of rocks, where predation by fish may not be possible. However, galaxiids may have stronger indirect effects on the abundance of nymphs because they are benthic fish. Morphological defences may also reduce predation by fish. *Coloburiscus* nymphs are heavily chitinized with many spines on their legs, abdomen and cerci. These structures may reduce the risk of predation by fish in streams, as discussed in Chapter Five.

Predatory invertebrates, *Archichauliodes* and *Polyplectropus,* were positively associated with *Coloburiscus* nymphs. Other predatory invertebrates were negatively associated, including *Stenoperla* and *Hydrobiosis*. Various possibilities exist to explain this. If *Coloburiscus* is preyed upon by *Archichauliodes* and *Polyplectropus* these predators may occur in streams where *Coloburiscus*, is also abundant. Conversely, low numbers of *Coloburiscus* nymphs may occur in streams where *Stenoperla* and *Hydrobiosis* nymphs are consuming them. However, the main prey of *Archichauliodes* and *Stenoperla* in the Glentui River were *Deleatidium* and chironomid larvae respectively (Devonport and Winterbourn, 1976), and despite being common *Coloburiscus* nymphs made up less than 1% of the gut contents of each species. Similarly, *Plectrocnemia* (same family as *Polyplectropus*) were found to feed mainly on chironomid larvae (Hildrew and Townsend, 1979). Therefore, it seems unlikely that these species are important predators of *Coloburiscus* nymphs. However, their large size and the presence of morphological defences in *Coloburiscus* may help explain this. The presence of spines on the abdomen, legs and cerci of *Coloburiscus* may deter predatory invertebrates from consuming nymphs.

4.4.2 *Abiotic Factors*

Channel stability is a major factor influencing the distribution and abundance of *Coloburiscus* in streams. This contention is supported by the results of the stream channel experiment, in which a lower abundance of nymphs was found in the disturbed treatment. *Coloburiscus* is a relatively large and cumbersome mayfly with legs and mouthparts specialized for filter-feeding. In contrast to more mobile mayflies, like *Deleatidium*, *Coloburiscus* moves very little (pers. obs.). Therefore, the chances of *Coloburiscus* surviving a disturbance are low, especially in streams where there is a high frequency of disturbances. In unstable streams, there is also constant removal of detritus (Winterbourn, 1997), which *Coloburiscus* nymphs rely on as food. Consequently, there may be less suitable food in unstable streams.

Two variables related to stream channel stability are velocity and substrate size. The size of *Coloburiscus* nymphs was positively associated with substrate size and negatively associated with current velocity. Larger substrates and slower velocities may equate to more stable conditions for larger *Coloburiscus* nymphs. Quinn and Hickey (1990) found that *Coloburiscus* abundance was positively associated with substrate size (up to large cobbles), and suggested that larger substrata may be associated with increased stability and greater water turbulence, which may lead to high oxygen concentrations (Quinn and Hickey, 1990). Therefore, the tendency for large nymphs to inhabit slower water and larger substrata may be a response to stream stability. However, trade-offs may exist between greater stability and food supply.

Current velocity is an important variable affecting filter-feeding invertebrates as it influences the transport of FPOM. At slower velocities, the supply of FPOM will be a reduced, but large *Coloburiscus* nymphs still inhabit slow water. The benefit of occupying more stable habitats therefore may override the benefits of a greater food supply. Nymphs are still able to feed at slow velocities but at very high velocities they may get dislodged from the substrate and not be able to feed at all. Substrate size also affects the retention of CPOM or FPOM in streams (Rounick and Winterbourn, 1983) and therefore indirectly influence food availability. Thus, larger substrates may trap more CPOM and FPOM, and increase the amount of food available to *Coloburiscus* nymphs.

Stream depth was also examined and was negatively associated with *Coloburiscus* size. Depth is often related to changes in velocity, with slower velocities in deeper sections of the stream. Therefore, larger *Coloburiscus* nymphs may tend to occupy shallower areas where velocity is higher. Larger nymphs may also move to shallower reaches of the stream prior to emergence.

4.4.3 Associations with other invertebrate species

Various invertebrate species were found to be positively or negatively associated with *Coloburiscus* nymphs in my stream survey. Negative associations with *Coloburiscus* nymphs included species from streams with poor water quality and low MCI scores (Stark, 1993) (e.g., *Oxyethira*, Muscidae, Acarina and *Austrosimulium*). *Coloburiscus* nymphs are not found in streams with these species because *Coloburiscus* is not tolerant of poor water quality. In contrast, positive associations were with species from streams with high water quality (e.g., *Nesameletus*, *Zephlebia*, *Oeconesus* and *Helicopsyche*). However, associations with other invertebrate species may relate to biotic interactions with *Coloburiscus*.

Positive associations with other invertebrate species could be expected if they affect the supply of seston to *Coloburiscus*. In this study, species positively associated with *Coloburiscus* nymphs may facilitate the transport of FPOM by their movements in the stream. For example, *Zelandoperla*, *Oeconesus*, *Zephlebia* or *Nesameletus* are all relatively large species which may move FPOM via their movements in the stream. Wallace et al. (1991) found that macroinvertebrates were responsible for moving significant amounts of FPOM in streams. Therefore, it is possible that other invertebrate species such as increase the supply of FPOM to *Coloburiscus*. As discussed previously, predatory invertebrates were also negatively and positively associated with *Coloburiscus*. However, further investigation would be required to fully understand associations between *Coloburiscus* and other invertebrate species.

CHAPTER

5

"Every animal has its enemies, and Nature seems to have taxed her skill and ingenuity to the utmost to furnish these enemies with contrivances for the destruction of their prey myriads. For every defensive device with which she has armed an animal, she has invented a still more effective apparatus of destruction and bestowed it upon some foe, thus striving with unending pertinacity to outwit herself..." Forbes (1887)

CHAPTER FIVE

Anti-predator Defences of *Coloburiscus humeralis*

5.1 INTRODUCTION

Predators induce a variety of anti-predatory responses in stream invertebrates. These can be an evolutionary response (i.e. morphological or chemical defences) or via ecological modification in space or time (i.e. predator avoidance) (see Kerfoot and Sih, 1987; Dodson et al., 1994). Studies of predator avoidance in some mayfly species imply alterations to drift are the main mechanism of predator avoidance (Poff, DeCino and Ward, 1991; Tikkanen, Muotka and Huhta, 1994; McIntosh and Townsend, 1994, 1995, 1996; Peckarsky, 1996; Peckarsky and McIntosh, 1998). However, mayfly nymphs may also emerge earlier and consequently at smaller sizes to avoid predatory trout (e.g. *Baetis*: Peckarsky, Taylor and McIntosh, 2002).

Morphological structures such as spines may also provide defence against predation by fish and predatory invertebrates. Many species show an increase in spine length in response to predatory fish, for example the predatory waterflea, *Bythotrephes longimanus* (Straile and Halbich, 2000) and some odonate larvae (Johansson and Samuelsson, 1994). Peckarsky (1996) also found that the anti-predatory behaviour of five mayfly species in response to two stonefly species varied between species. Some used morphological defences, whereas others relied on movement. The fast moving *Baetis* sp. avoided predators by drifting at night, whereas

Ephemerella showed no escape behaviour from either stonefly species and used spiny cerci to ward off predators with a "scorpion" posture instead.

The evolution of inducible defences, like those discussed above, are dependent on four factors (Harvell and Tollrian, 1999). The amount of selection pressure for a particular trait must be variable, there must be a reliable cue to induce the defence, there must also be some "cost saving" for having an inducible defence (rather than a fixed defence), and finally the defensive structure must be effective (Harvell and Tollrian, 1999). Therefore, trade-offs exist between the immediate benefits of predator avoidance and the long term costs of maintaining anti-predator defences or behaviours (Lima, 1998).

Coloburiscus nymphs have a heavily chitinized thorax, spiny gills, and spikes and bristles on the cerci (Wisely, 1961). The purpose of these structures is unknown but it is likely that they provide defence against predation. Peckarsky (1996) argued that mayflies more adapted to a sedentary lifestyle were more likely to use morphological defences. *Coloburiscus* nymphs are commonly found under rocks or in crevices (Wisely, 1962) and movement is limited. The spines, bristles and cerci may be used to reduce the risk of predation by fish and invertebrates making a more sedentary life-style possible.

The same morphological structures may be employed for other functions. The nymphs of *Coloburiscus* are associated with fast flowing sections of streams, the under surfaces of rocks or they may be attached to mossy vegetation. The spines on the legs of *Coloburiscus* may be used to help cling to vegetation and rocks. It has also been suggested that the spiny gills of the morphologically similar *Coloburiscoides* may function to maintain position in the stream current (Campbell, 1985). As *Coloburiscus* and *Coloburiscoides* are morphologically similar it is possible that the gills are also used for this purpose in *Coloburiscus* nymphs.

This chapter examines the morphological characteristics of *Coloburiscus* in response to different predation regimes, including predatory fish and predatory invertebrates. In Chapter Three, predatory galaxiids were found to significantly affect gut fullness of *Coloburiscus* nymphs but few nymphs were consumed in the predation experiment (Chapter Four). It is possible that morphological defences lower the risk of predation by predatory fish. Risk of predation by predatory

invertebrates may also be a factor for *Coloburiscus* nymphs if they share the same habitat. In addition to fish, the presence of *Archichauliodes* was examined with respect to morphological defences as it is often associated with *Coloburiscus* (see Chapter Three). *Archichauliodes* is also one of the only predatory invertebrates large enough to be a potential threat to *Coloburiscus*.

5.2 METHODS

Invertebrate samples were taken from eight streams in the Cass-Craigieburn region of the Southern Alps and five streams on Banks Peninsula. These sites are described in detail in Chapter Three. Every individual within each sample was counted and five morphological measurements were made. They included head capsule width, body and cercal lengths, abdominal segment VII spine length and leg spine length (Fig. 5.1).

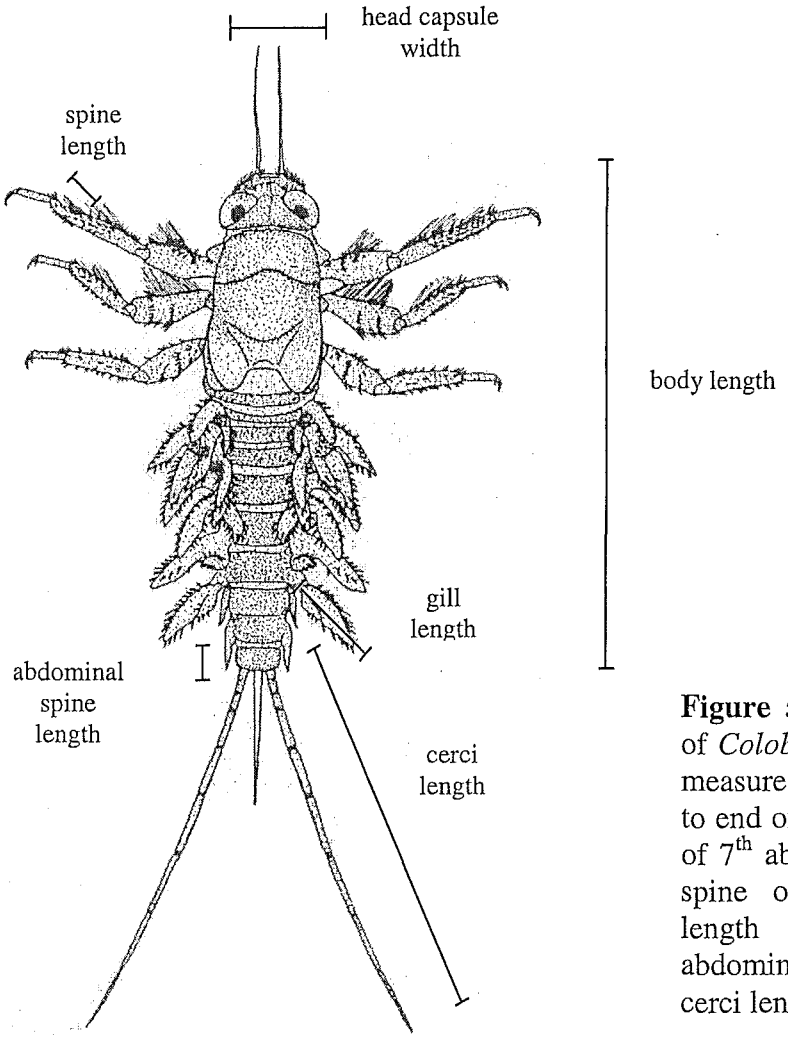

Figure 5.1: Body length of *Coloburiscus humeralis* measured from tip of head to end of abdomen. Spine of 7th abdominal segment, spine on tibiae of leg, length of gill on 6th abdominal segment and cerci length measured.

5.2.1 Statistical Methods

The morphological measurements taken were all divided by body length to exclude size as a factor. Four response variables were used in this analysis: cercal length, abdominal spine length, gill length and leg spine length. Stream means were used as replicates in an ANOVA to test the effect of predatory fish on the morphological characteristics of *Coloburiscus* nymphs. Region was initially included as a blocking variable to examine differences related to the areas sampled. However, region had no significant effect and was removed from the analysis. A two factor ANOVA was then used to test the effects of predatory trout and galaxiids in a factorial design on the four response variables.

The effect of *Archichauliodes* on morphological characteristics of *Coloburiscus* nymphs was also tested using an ANOVA with region as a blocking term. In this analysis, region was left in the model for leg spine length, but was removed from the ANOVA investigating gill length, cercal length and abdominal spine length.

5.3 RESULTS

5.3.1 Morphological Defences of Coloburiscus humeralis

Coloburiscus nymphs are potentially well defended from predation by a multitude of spines on the gills, abdomen and legs. During observations made on the feeding behaviour of *Coloburiscus* in Chapter Two, nymphs were observed posturing with their cerci. The cerci was raised above the body and rotated in a circle for a short period of time. *Coloburiscus* nymphs were also observed to "play dead" when introduced into the observation aquarium and when touched by passing invertebrates.

5.3.2 Predatory Fish

Presence of predatory fish in a stream did not affect the length of abdominal spines, gills or leg spines in *Coloburiscus* nymphs from Banks Peninsula and Cass (Table 5.1). There was little variation in the length of these morphological

characteristics between streams containing different fish (Fig. 5.2 a - c). Predatory fish, however did significantly affect the length of cerci (Table 5.1). Nymphs from fishless streams had the longest cerci (4 – 7 mm) and streams with only galaxiids present had the shortest (3 – 5 mm) (Fig. 5.2 d). There was a significant interaction between trout and galaxiids (Table 5.1) indicating that when trout were present, galaxiids did not have such a large effect on the length of the cerci of *Coloburiscus* nymphs. Nymphs from streams with only galaxiids had significantly shorter cerci than nymphs from streams with trout and galaxiids (Fig 5.2 d).

5.3.2 Predatory Invertebrate – Archichauliodes diversus

The predatory invertebrate, *Archichauliodes diversus* had variable effects on the four morphological characteristics of *Coloburiscus humeralis* measured. Gill and abdominal spine length were not significantly different in streams with and without *Archichauliodes* (Table 5.2, Fig. 5.3 a and b). Leg spine length of *Coloburiscus* nymphs was significantly different between the two regions (Table 5.2). Larger leg spines occurred in nymphs from Cass than in nymphs from streams on Banks Peninsula (Fig. 5.3 c). In both regions, the spines on the legs of *Coloburiscus* nymphs were longer when *Archichauliodes* was present (Fig. 5.3c). The length of *Coloburiscus* cerci was not significantly different when *Archichauliodes* were present or absent (Table 5.2, Fig. 5.3 d).

Table 5.1 Analysis of Variance testing the factorial effects of the types of predatory fish (trout present or absent and galaxiids present or absent) on (a) cercal length, (b) gill length, (c) abdominal spine length and (d) leg spine length of *Coloburiscus humeralis* (ln transformed data).

Source	(a) Cerci Length				(b) Gill Length			
	df	MS	F - ratio	*p*	df	MS	F - ratio	*p*
Galaxiid	1	0.022	1.104	0.321	1	0.001	0.043	0.840
Trout	1	0.001	0.047	0.833	1	0.008	0.557	0.474
Trout x Galaxiid	1	0.113	5.710	0.041	1	0.011	0.771	0.403
Residual	9	0.020			9	0.014		

Source	(c) Abdominal Spine Length				(d) Leg Spine Length			
	df	MS	F - ratio	*p*	df	MS	F - ratio	*p*
Galaxiid	1	0.000	0.013	0.910	1	0.004	0.085	0.777
Trout	1	0.004	0.366	0.560	1	0.022	0.514	0.492
Trout x Galaxiid	1	0.001	0.117	0.740	1	0.000	0.005	0.946
Residual	9	0.012			9	0.042		

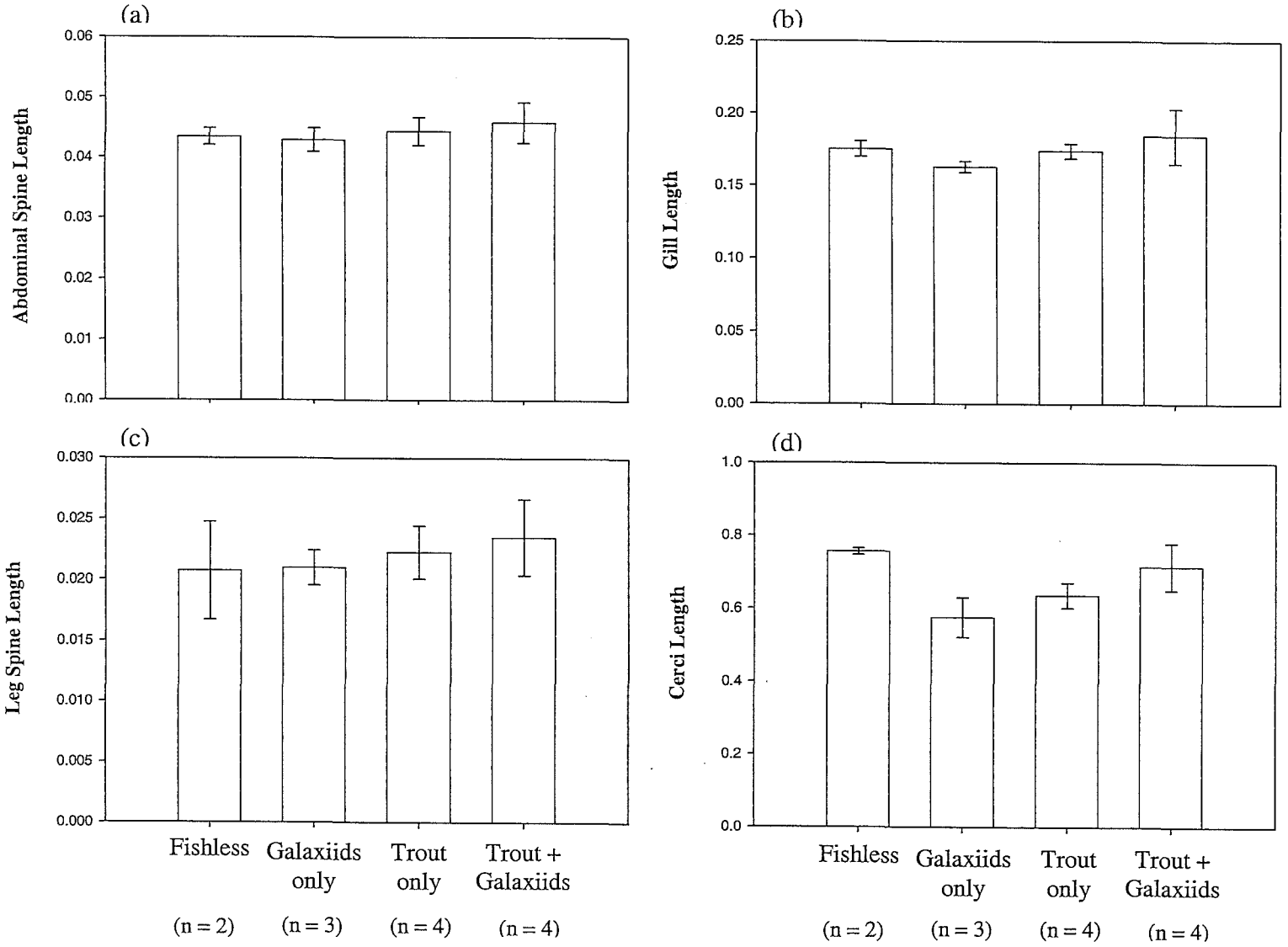

Figure 5.2: Mean (± 1 S.E.) length of (a) abdominal spine, (b) gill, (c) leg spine and (d) cerci of *Coloburiscus humeralis* nymphs in relation to the presence of predatory trout and galaxiids. Numbers below x-axis labels indicate the number of replicate streams sampled for each treatment. Values plotted are the means of the average values calculated from all individuals measured per stream and are in mm units standardised by total length (e.g. cerci length (mm) / total length (mm)).

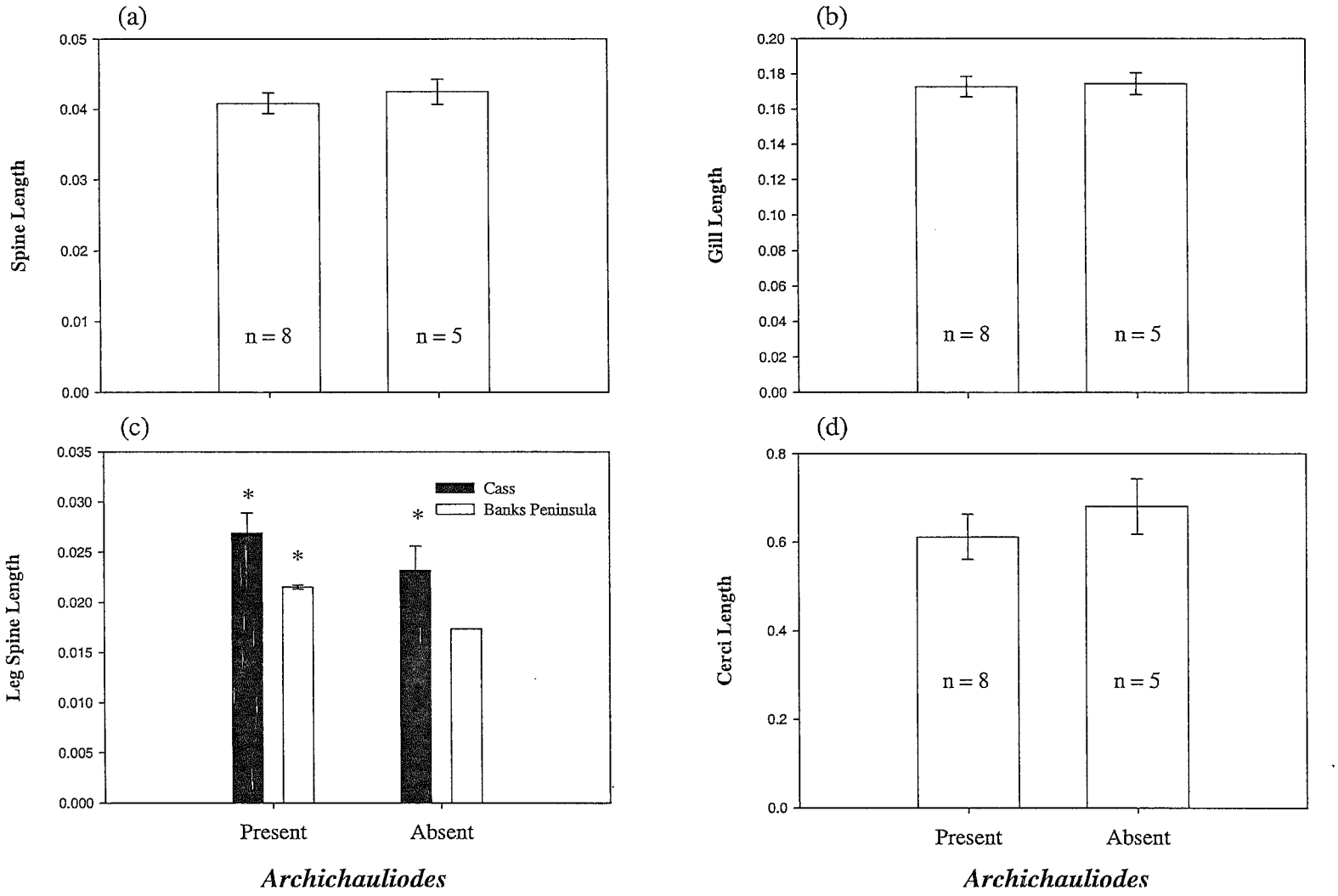

Figure 5.3: Mean (± 1 S.E.) standardised lengths of (a) abdominal spine, (b) gill, (c) leg spine and (d) cerci of *Coloburiscus humeralis* nymphs in relation to the presence of a predatory invertebrate, *Archichauliodes diversus*. Values plotted are the means of the average values calculated from all individuals measured per stream. "*" indicates four replicate steams sampled for each treatment; unlabeled bar indicates one replicate only.

Table 5.2: Results of Analysis of Variance (ANOVA) testing the effects of predatory invertebrates and region on (a) cercal length, (b) gill length, (c) abdominal spine length and (d) leg spine length of *Coloburiscus humeralis* (ln transformed data).

Source	(a) Cerci Length				(b) Gill Length			
	df	MS	F - ratio	*p*	df	MS	F - ratio	*p*
Archichauliodes	1	0.042	0.717	0.415	1	0.000	0.044	0.838
Residual	11	0.058			11	0.008		

	(c) Abdominal Spine Length				(d) Leg Spine Length			
	df	MS	F - ratio	*p*	df	MS	F - ratio	*p*
Archichauliodes	1	0.005	0.507	0.491	1	0.082	4.680	0.056
Region	-	-	-	-	1	0.151	8.558	0.015
Residual	11	0.010			10	0.018		

5.4 DISCUSSION

Predators affect the distribution and abundance of some prey species (Kerfoot and Sih, 1987). However, predators can also alter the behaviour of their prey and induce morphological defences to reduce predation (Edmunds, 1974). Anti-predator defences are widely known in nature (see Edmunds, 1974) and studies of this phenomenon in freshwater ecosystems are becoming more common (Peckarsky, 1980; Scrimgeour, Culp and Cash, 1994; Straile and Halbich, 2000). In this chapter, four morphological characteristics of *Coloburiscus* nymphs were examined in response to the presence of predatory fishes and a predatory invertebrate. Two of these morphological traits (cerci and leg spine length) showed a response to the presence of predators.

5.4.1 Predatory Fish

Cercal length of *Coloburiscus* nymphs was smaller in streams with predatory galaxiids than in streams with no fish or trout. This contrasts with the results of past studies where the defensive structures of prey species are often larger when predatory species are present (Johansson and Samuelsson, 1994; Straile and Halbich, 2000). *Coloburiscus* nymphs were observed to posture by raising and rotating the cerci, which may be an anti-predator behaviour. The cerci of mayfly and stonefly nymphs have been proposed as anti-predator defence structures (Peckarsky, 1980, 1987; Otto and Sjöström, 1983). *Ephemerella*, a North American mayfly, responds to predation by freezing and posturing with its cerci (Peckarsky, 1980). This behaviour may alter the perception of its predators by making it look larger than it really is and increase the effectiveness of the abdominal spines (Peckarsky, 1987). Similar patterns have been observed in stoneflies, where attacks by fish from the front of nymphs resulted in posturing (Otto and Sjöström, 1983). *Coloburiscus* nymphs in streams with predatory galaxiids may use this posturing behaviour to reduce the risk of predation and in the process the cerci may get broken off.

When trout and galaxiids were both present in a stream, the cerci of *Coloburiscus* were longer than when only galaxiids were present. The best

explanation for this is that trout may be reducing the amount of predation pressure galaxiids exert on *Coloburiscus*. Trout are known to restrict the activity of some galaxiids (McIntosh et al., 1992; Edge et al., 1993; also see Chapter Three discussion). The cerci may still be used as defence against trout although because they are mainly drift feeders (McIntosh and Townsend, 1995), encounters with trout may be less frequent than encounters with benthic galaxiids.

Spines and cerci may reduce the risk of actually being consumed. Trout have been observed to eject more stonefly nymphs with intact cerci after initial consumption than nymphs without cerci (Otto and Sjöström, 1983). Trout have also been observed to ingest *Coloburiscus* nymphs and then to quickly egest them (C. Bell, pers. comm.). Therefore, cerci may act as a "secondary" defence and prevent predatory fish from consuming *Coloburiscus*.

Cerci are present (unless broken off) on *Coloburiscus* whether predatory fish are present or not, but their length may change in response to predation pressure by fish. In this study, cerci were shorter when predatory fish were present. It may be that encounters with predatory fish result in cerci getting broken. Therefore, there is obviously a cost to not having cerci but are there benefits to be had from having long or short cerci?

Two of the criteria for having inducible defences are: (1) that the structure must be effective as a defence mechanism and (2) there must be some cost that offsets the benefit of having the structure, otherwise it would be permanent (Harvell and Tollrian, 1999). The growth of spina in the planktonic waterflea, *Bythotrephes longimanus* was found to increase with predation pressure by fish (Straile and Halbich, 2000). Similarly, abdominal spine length was found to increase in response to fish predators in *Leucorrhinia dubia* (Johansson and Samuelsson, 1994). In these two invertebrate species, spines may be effective defences against predators, however, they may be energetically costly to produce so they are inducible defences. In *Coloburiscus*, the presence of cerci may alone deter predators.

Peckarsky (1987) suggested that cerci increased the apparent size of mayfly nymphs. Therefore, it may be beneficial for *Coloburiscus* to have longer cerci when predatory fish are present since it increases the perceived size of nymphs and consequently reduces predation. However, inducible defences are favoured when the

risk of predation is variable (Harvell and Tollrian, 1999) Almost all New Zealand streams contain predatory fish or have fish in their downstream reaches. Therefore, although there may be costs associated with producing long cerci they may always be beneficial because there is a constant risk of predation by fish. Dahl and Peckarsky (2002) found that longer caudal filaments increased the chances of survival of *Drunella* against predatory trout compared to mayflies with artificially shortened cerci. However, only those mayflies from fishless streams produced longer cerci in response to predatory fish (Dahl and Peckarsky, 2002). Therefore, cercal length in *Coloburiscus* may be an evolutionary response to the presence of predatory galaxiids in streams and nymphs may no longer respond to the presence of fish. Cerci may also be used to maintain position in the substrate, provide sensory information (Otto and Sjöström, 1983), or be used by adults in flight (Hynes, 1970).

The other three morphological characteristics measured (gill, leg spine and abdominal spine length) were not significantly different between fish treatments. The very small amount of variation in the size of these structures suggests that they probably don't respond to changes in predation risk by fish (Fig. 5.2). These structures, like the cerci, may be permanent defensive structures as the risk of predation by fish is constantly high. The gills are also used in respiration and the spines associated with them may serve other functions, such as preventing the accumulation of particulate material on the respiratory surfaces.

5.4.2 Predatory invertebrate – Archichauliodes diversus

The characteristic under-rock habitat of *Coloburiscus* nymphs may reduce the risk of predation by fish, and consequently the risk of predation by predatory invertebrates may be more common. Cercal length showed the greatest variation in length but was not significantly affected by the presence of *Archichauliodes* (Fig. 5.3). *Ephemerella* used its cerci to deter stonefly predators in a North American study (Peckarsky, 1987). However, the same stonefly predators were deterred when *Ephemerella* did not posture with its cerci, indicating that other factors may also be important as anti-predator mechanisms. Abdominal spine length and gill length were not significantly affected by the presence of *Archichauliodes* in streams and may be

permanent defensive structures. However, the gills primary function is probably not defence.

The legs of *Coloburiscus* are covered in large spines, the size of which was found to be significantly different in the presence of *Archichauliodes* and between regions. At both Cass and Banks Peninsula, there was a trend for smaller spines to be found on nymphs from streams without *Archichauliodes* present. *Coloburiscus* may use leg spines to prevent or reduce predation by *Archichauliodes*. The spines on the legs may be more effective against an invertebrate predator, like *Archichauliodes,* than the cerci because they are easier to manipulate against a predator that can be about the same size of *Coloburiscus*. The spines on the legs may be considered inducible defences as they varied with the presence of the predatory invertebrate, *Archichauliodes*, the spines would also be costly to produce.

5.4.3 Multiple Predators

The production of larger defensive structures in the presence of predatory fish and invertebrates is common in invertebrates (see Tollrian and Harvell, 1999). However, the effect of multiple predators on the production of morphological defences is relatively unknown. In this study, the presence of predatory galaxiids was correlated with changes in cercal length of *Coloburiscus* nymphs. However, when *Archichauliodes* was present *Coloburiscus* had longer spines on the legs. Therefore, there must be additional pressures when two types of predators are present. In other words, do defences against one predator lead to increased vulnerability to another?

Coloburiscus may develop different defences against different predators in the same way that *Daphnia* responds differently to different predators by developing an elongated tailspine, helmet and/or increased body size (Lüning, 1993; Schulz and Yurista, 1999; Barry, 2000). Producing two different defences against two predators may be energetically costly, therefore it would be beneficial for *Coloburiscus* to develop a defence that may be used against both predatory invertebrates and fish. Further research is required into the possible effects of multiple predators on the morphological defences of *Coloburiscus* in streams.

CHAPTER 6

DISCUSSION

CHAPTER SIX

General Discussion

In this study, the ecology of a species and the factors controlling its distribution and abundance were examined. To understand the distribution and abundance of a species we need to know about an organism's evolutionary history, the resources it requires, the rate of birth, death and migration, interactions with conspecifics and other species, and finally the effects of environmental conditions on the species (Begon, Harper and Townsend, 1996). Previous studies of *Coloburiscus humeralis* have documented various aspects of its life history (Wisely, 1961, 1962, 1965; Harding and Winterbourn, 1993). The purpose of this thesis was to expand on these studies and examine the distribution and abundance of *Coloburiscus humeralis* populations with respect to its abiotic and biotic environment.

Coloburiscus show many adaptations for life in stream environments, including specialised morphological adaptations for filter-feeding. The prominent hairs on the legs and mouthparts function to capture food particles and allow *Coloburiscus* nymphs to passively filter feed in a variety of flowing water habitats. In comparison to grazing invertebrate species whose food source is fixed, *Coloburiscus* nymphs have a relatively "mobile" food source. Consequently, nymphs may select sites where filter-feeding is most profitable.

6.1 Factors controlling the distribution of *Coloburiscus humeralis* in streams

6.1.1 Abiotic Variables

Organisms are limited by their physiological tolerance to a range of environmental conditions, for example temperature and oxygen concentration (McAuliffe, 1983). In New Zealand streams the distributions of many invertebrates are controlled by physical factors (Winterbourn et al., 1981; Quinn and Hickey, 1990b; Jowett and Richardson, 1990). This was also the case for *Coloburiscus* in three regions of the South Island, where physical factors primarily controlled the distribution of nymphs. *Coloburiscus* nymphs were generally not present in unstable streams and showed distinct preferences for substrate size, velocity and depth at different sizes. The in-stream channel experiment also supported the conclusion that disturbance excluded *Coloburiscus* from streams.

Abiotic variables associated with the abundance of *Coloburiscus* in streams generally relate back to stream stability. For example, preferences for larger substrata and slow to medium current velocities. *Coloburiscus* is a large mayfly, which lives a relatively sessile life-style. Therefore, nymphs may prefer areas where there is less chance of mortality due to disturbance.

6.1.2 Biotic Variables

Predatory fish were found to have little effect on the distribution and abundance of *Coloburiscus* nymphs in streams near Cass and Hanmer and on Banks Peninsula. The channel experiment also showed that predatory fish were not consuming nymphs. However, native galaxiids appeared to have an impact on the feeding behaviour of *Coloburiscus* at night, possibly by limiting the feeding of nymphs. Predatory galaxiids may also restrict the activities of other invertebrates in the stream, thereby reducing the transport of FPOM, although this was not demonstrated in this study.

Morphological defences, such as spines or cerci, may prevent predatory fish and invertebrates from consuming *Coloburiscus*. In streams with predatory galaxiids, *Coloburiscus* nymphs had shorter cerci. However, the cause of this is unknown,

although *Coloburiscus* may use its cerci to prevent predation and in the process the cerci may get broken. Morphological defences responded differently when predatory invertebrates were present. Spines on the legs were longer when *Archichauliodes* was present, which suggests these structures may be used as inducible defences against predatory invertebrates.

The response of *Coloburiscus* to food supply was the opposite of that expected, with nymphs having less in their guts when there was more organic and total (organic and inorganic) seston available. Again the cause of this is unclear but it is possible that high amounts of seston were present in streams with predatory galaxiids. In these streams, the feeding activity of nymphs may have been reduced due to the presence of predatory galaxiids. However, it is also possible that at higher food concentrations, the gut passage time of nymphs is reduced and because they are processing the food faster less food appears to be in the guts.

6.2 Models explaining invertebrate distribution and abundance

Many models exist to explain patterns in the distribution and abundance of organisms (e.g. top-down or bottom-up control). In this section, two will be considered: the "harsh-benign model" proposed by Peckarsky (1983) and the "multitrophic model" proposed by Wootton et al. (1996).

6.2.1 "Harsh-benign model"

Peckarsky (1983) suggested that interactions between invertebrate community structure and abiotic and biotic factors are dependent on harshness of physical conditions. The following model was proposed to explain patterns of invertebrate community structure in North American streams (Fig. 6.1).

When physical conditions are benign, a large predator population can exist and may limit prey population numbers (Fig. 6.1). This in turn reduces competition for space in the prey species. When the effectiveness of predation is reduced for example, via prey defences, changes in the physical environment or increased refugia from predation, competition for food or space may become more prevalent

(Peckarsky, 1983). However, when physical conditions become increasingly harsh, community structure may be determined by physical factors rather than biological factors (i.e., predation or competition) (Fig. 6.1) (Peckarsky, 1983).

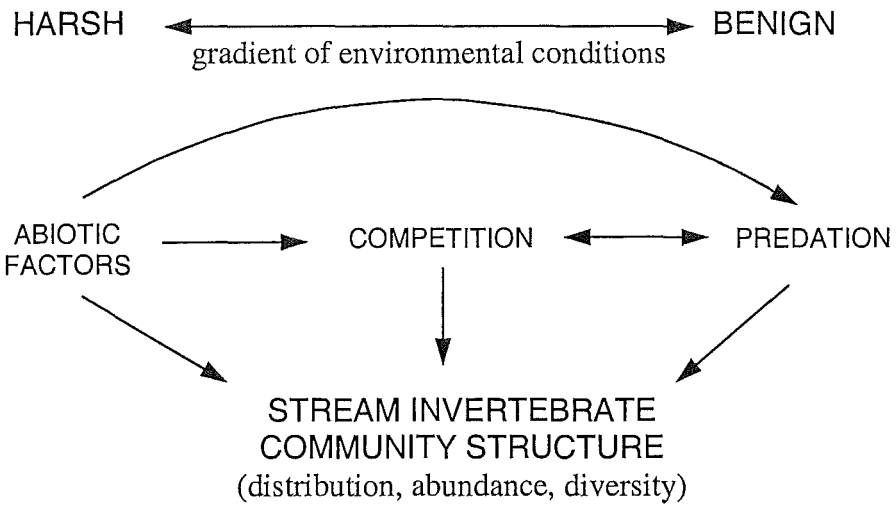

Figure 6.1: Hypothetical model proposed by Peckarsky (1993) to explain invertebrate community structure.

6.2.2 "Multitrophic model"

This model predicts that the removal of flood disturbances will increase the number of predator-resistant invertebrates, which in turn alters the amount of energy available to predator-susceptible invertebrates (Wootton et al., 1996). For example, large predator-resistant species will be more susceptible to disturbance than mobile predator-susceptible invertebrates because they have invested more energy into avoiding predators (Fig. 6.2, Wootton et al., 1996). Wootton et al. (1996) found that the abundance of the predator-resistant caddisfly, *Dicosmoecus*, increased in the absence of floods. Factors which contribute to increased defence from predators (large body size and protective case), increased the susceptibility of this invertebrate to disturbance. This resulted in changes in availability of algae to predator-susceptible species, i.e. increased abundance of *Dicosmoecus* resulted in a decreased supply of algae (Wootton et al., 1993).

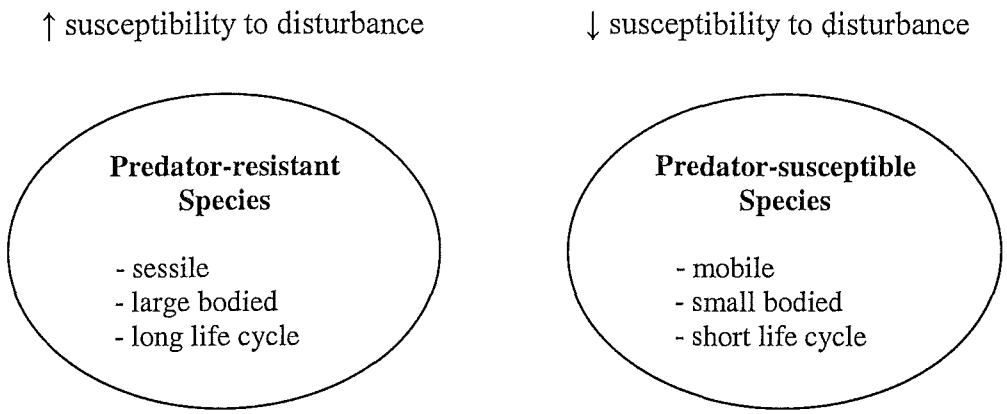

Figure 6.2: Model proposed by Wootton et al. (1996) explaining patterns of distribution in stream invertebrates.

6.2.3 Implications for Coloburiscus *in New Zealand streams*

Mayflies have been found to invest in a variety of anti-predator tactics (Peckarsky, 1996). The response of five North American mayfly species varied depending on initial investment in morphological defences. For example, the morphologically defended *Ephemerella* did not move in response to the presence of predatory fish, however *Baetis* which has no observed morphological defences relied heavily on its fast movement to avoid predation (Peckarsky, 1996). In New Zealand streams, *Nesameletus* and *Deleatidium* do not possess any morphological defences, however by being able to move fast they can avoid predation. In contrast, *Coloburiscus* possesses many morphological defences to prevent predation by fish but is a slow mover.

The results of this study support the model proposed by Peckarsky (1993). New Zealand streams are relatively harsh environments for stream invertebrates (Winterbourn et al., 1981) and therefore the fact that physical factors control the distribution and abundance of *Coloburiscus humeralis* in streams is not surprising. However, contrary to the "harsh-benign model", predation does have some effect on *Coloburiscus* nymphs. Therefore, there is support for the theory that a trade-offs exist between susceptibility to predation and disturbance.

As with *Dicosmoecus* in the Wootton et al (1996) study, *Coloburiscus* is susceptible to disturbance because of its large size, and morphological adaptations for feeding and anti-predator defence. *Coloburiscus* nymphs also spend between 1 - 2 years in streams (Harding and Winterbourn, 1993), which increases the length of time it is at risk from predation. Therefore, it may be more important for *Coloburiscus* to invest in anti-predator strategies than "anti-disturbance" strategies. The main evidence I obtained that supports the "multitrophic model" is that *Coloburiscus* is not found in disturbed streams, and that nymphs are not present in large numbers in the diet of predatory fish.

6.3 Conclusion

As mentioned at the beginning of this thesis, "ecology is not a science with simple linear structure: everything affects everything else" (pg vii, Begon et al., 1996). The results of this study suggest that trade-offs exists between vulnerability of *Coloburiscus* to predation and disturbance. *Coloburiscus* were not found in unstable streams and morphological defences reduced the susceptibility to predation. Therefore, although seeming unrelated, these factors appear to control the distribution and abundance of *Coloburiscus* in streams. Factors related to these variables, such as substrate size and velocity, are also important in determining the distribution of *Coloburiscus humeralis* in streams.

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